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STUDIES ON THE CIRCULATION IN MAN.

XII. A Study of Inequalities in the Blood Flow in the Two Hands (or Feet) Due to Mechanical Causes (Embolism, Compression of Vessels, Etc.) or to Functional (Vasomotor) Causes, with a Discussion of the Criteria by Which the Conditions Are Discriminated.

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In this paper a few typical cases are discussed to illustrate the criteria referred to in the title. Other cases presenting inequalities in the flow are included in Paper XIII of this series,[1] under unilateral peripheral neuritis and hemiplegia. Some have been published in previous papers, and of these one or two will be briefly alluded to. The flow observations are summarized in Table I.

A criterion which could be predicted *a priori* for a blood flow diminished by a mechanical obstruction would be the approximate constancy of the ratio of the flow in the obstructed part to that in the corresponding normal part in measurements made at such short intervals that collateral circulation could not be appreciably increased in the interval. If the obstruction is so great that the chief resistance of the vascular path is situated there, it is clear that a further criterion would be the lack of any marked response of the blood flow in the part to vasoconstrictor or vasodilator influences; *e. g.*, to reflex vasomotor effects from the contralateral part. It will, of course, depend upon the degree of the obstruction and the extent to which a collateral circulation has been opened up how great an effect on the flow will be produced through the vasomotors. Under no circumstances could we expect the ratio of the flow in the

[1] Stewart, G. N., *Arch. Int. Med.*, 1915, xvi (in press).

obstructed part to that in the normal part to be increased by conditions favoring vasodilatation acting equally on both parts or on the whole surface, for example, by an increase of the air temperature, whereas general cutaneous vasodilatation might very well alter the ratio to the disadvantage of the affected part. On the other hand, purely central changes, affecting only the driving power of the heart, would leave the ratio unchanged, however great the absolute changes in the flows might be.

These considerations have been verified in a number of cases. One of these has been already described.[2] The innominate and right common carotid arteries were ligated by my colleague, Dr. Carl Hamann, in a woman 68 years old. for subclavian aneurysm. About a month after the operation the ratio of the flow in the right hand to that in the left was 1 : 3.54 and 1 : 3.48 on two successive days. The stability of the ratio, although the flow in both hands was somewhat greater at the first than at the second examination, is obvious. Also practically no vasodilatation was caused in the right hand when the left was immersed in warm water. The stability of the ratio is clearly, and the failure of the contralateral reflex to influence the flow is almost certainly, associated with the still very effective mechanical block on the arterial path of the limb.

Sixteen weeks later the collateral circulation had opened up so well that the ratio had increased to 1 : 1.3, and the vasomotor reflexes from the left to the right hand were now distinctly shown by the change in the blood flow.

A case with a different kind of mechanical block, namely, embolism (and thrombosis) in the left arm and the right leg, illustrates equally well the criterion of stability of the ratio of the flows. There was apparently at one time some temporary plugging of vessels in the right arm also, but this cleared up.

Costa B., a dyer, aged 47 years, was in Lakeside Hospital from Apr. 13, 1914, to May 9, 1914, suffering from rheumatic fever, and left with the physical signs of mitral stenosis and insufficiency. On July 31, 1914, he was readmitted to the hospital. That morning while at work he suddenly felt a severe pain in the right leg, worst in the groin. No pulsation could be detected in the right dorsalis pedis. The anterior tibial pulse could be felt, but was very feeble. No pulse in the right popliteal. Good pulsation in the left leg in all accessible arteries. No pulse in the left radial artery, but the right is strong, rhythmic, regular, and well sustained. The vessel wall is palpable, but not nodular. A strong pulse can be felt in the right brachial. Blood pressure, left arm, systolic 114, diastolic 85.

Sept. 26, 1914. The pulse is obliterated in the left radial. The left hand is cold and pains him. Sept. 30. He says there is tingling of the left hand and right foot. The left radial pulse is absent and never returned after this. The right radial pulse is diminished. Right brachial felt.

Oct. 3, 1914. He says that there is no discomfort except tingling in the right foot. Both extremities are warm. The right femoral pulse can be felt, but not the dorsalis pedis. No pulse in the left radial or brachial. Pulse in

[2] Stewart, *Arch. Int. Med.*, 1913, xii, 678.

right radial and brachial. On Oct. 5 it is stated that the right radial pulse could not be felt. On Oct. 8 the right radial pulse was again felt, and was always present thereafter. On Oct. 10 he complains of pain in the left shoulder and down the arm to the elbow. Physical signs of (compensated) mitral stenosis and insufficiency. The blood flow was examined on Oct. 26, 1914, at which time no improvement had occurred in the permeability of the vessels which could be detected by palpation. The left foot is warmer than the right to the touch.

First Examination of Blood Flow.—Costa B., Oct. 26, 1914. Hands in bath at 2.56 p.m., in calorimeters at 3.07, out of calorimeters at 3.28. As always, unless otherwise mentioned, the quantity of water in each hand calorimeter was 3,015 cc.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.06½	31.63	31.53		3.18	31.72	31.435	
3.08	31.59	31.48		3.19	31.75	31.43	22.4
3.09	31.595	31.48	21.4	3.20	31.78	31.425	
3.10	31.61	31.475		3.21	31.80	31.42	
3.11	31.62	31.47	21.3	3.22	31.82	31.41	22.6
3.12	31.62	31.46		3.23	31.84	31.405	
3.13	31.63	31.46	21.4	3.25	31.895	31.40	
3.14	31.65	31.455		3.26	31.92	31.40	
3.15	31.67	31.45	21.7	3.27	31.945	31.395	
3.16	31.695	31.455		3.28	31.98	31.385	22.8
3.17	31.71	31.44	22.0	3.41	31.79	31.22	

Cooling of calorimeters in 13 minutes, right 0.19°, left 0.165°. Volume of right hand 473 cc., of left 441 cc. Water equivalent of calorimeters with contents, right 3.473, left 3.448. Pulse in carotid 102.

Feet in bath at 3.44½ p.m., in calorimeters at 3.55½, and out of calorimeters at 4.24. As always, unless otherwise mentioned, the quantity of water in each foot calorimeter was 2,775 cc.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.55	32.17	32.19		4.14	31.69	31.95	22.0
3.57	32.03	32.06	21.9	4.16	31.68	31.97	
3.59	31.97	32.00		4.18	31.67	31.99	22.0
4.01	31.90	31.96	22.0	4.20	31.66	32.02	
4.03	31.85	31.93		4.22	31.65	32.04	22.05
4.05	31.78	31.91	22.0	4.24	31.64	32.06	
4.07	31.76	31.91		4.26	31.59	32.03	
4.09	31.73	31.91	22.0	4.34	31.40	31.84	22.1
4.12	31.70	31.93					

Cooling of foot calorimeters, 0.19° in 8 minutes for right and left. Volume of right foot 1,191 cc., of left 1,188 cc. Water equivalent of foot calorimeters with contents, right 3,856, left 3,854. Rectal temperature 37.45° C.

The blood flow in the hands for the last fifteen minutes in the calorimeters was 6.03 gm. per 100 cc. per minute for the right hand, and 1.28 gm. for the left, with room temperature 22° C. The ratio of the flow in the left foot to that in the right was 1 : 4.71. For the feet the flows were 1.25 gm. per 100 cc. per minute for the right, and 2.50 gm. for the left, with room temperature 22° C. calculated for the last 15 minutes in the calorimeters when the flows had become steady (ratio 1 : 2). The ratio of the sum of the foot flows to the sum of the hand flows was 1 : 1.94, and the ratio of the flow in the normal (left) foot to that in the normal (right) hand, 1 : 2.41, both within the normal range for the ratio of foot to hand flow.

The man was discharged on Nov. 20 from Lakeside Hospital, and was admitted at the City Hospital on Feb. 2, 1915. He complains of pain in the right leg from the groin down. When he walks a little, the pain gets worse. There are also shooting pains down the leg. On placing a constricting band on the left arm, the superficial veins filled slowly. When the band was put on the right leg, none of the superficial veins filled. The veins of the left leg and right arm filled rapidly (Feb. 16). On Feb. 9 the blood examination gave erythrocytes 5,100,000, leucocytes 6,400. Wassermann negative. Blood pressure (Feb. 9), systolic 95, diastolic 65; (Feb. 11) 110 and 75. On admission the boundaries of the cardiac dullness were the third rib, the right sternocostal margin, and 2 cm. to the left of the nipple line. The blood flow was examined on Feb. 24, 1915. At this time the grip of the left hand was strong, scarcely weaker than that of the right. The right foot and lower leg feel markedly cold to the touch, and there is no pulse in the dorsalis pedis or elsewhere in the leg. No pulse at the left wrist, and the left hand is colder to the touch than the right. There is no difference in the nails of the two hands. He complains of pains in the right leg and says that it feels tired all the time. The left hand does not now trouble him much. There is some wasting of the right leg.

Second Examination.—Feb. 24, 1915. Hands in bath at 3.12 p.m., in calorimeters at 3.22, and out of calorimeters at 3.35.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.21	31.89	31.82		3.31	32.09	31.87	
3.23	31.85	31.80	23.8	3.32	32.12	31.88	23.8
3.24	31.88	31.82	23.8	3.33	32.16	31.89	
3.25	31.90	31.825		3.34	32.195	31.895	
3.26	31.93	31.83		3.35	32.26	31.90	23.9
3.27	31.97	31.84	23.8	3.55	32.06	31.67	
3.29	32.03	31.86	23.7				

Cooling of calorimeters in 20 minutes, right 0.20°, left 0.23°. Volume of right hand 498 cc., of left 452 cc. Water equivalent of calorimeters with con-

tents, right 3,493, left 3,457. Pulse 81. The right radial pulse was surprisingly difficult to feel considering the fair blood flow.

The blood flow for the right hand at this examination was 6.96 gm. per 100 cc. per minute, and for the left hand 2.54 gm. (for 11 minutes in the calorimeters), with room temperature 23.8°. For the right hand this is practically the same as on Oct. 26, 1914, four months earlier, considering the higher room temperature at the second examination. In the left hand, however, the flow is twice as great as on the first occasion, so that the ratio of the hand flows is now 1 : 2.74, indicating a great improvement in the collateral circulation in the left hand. This is quite in accordance with the general condition of the hands. There is, of course, still a decided mechanical obstruction on the left side, and the new ratio of the hand flows is stable for successive measurements made at short intervals, as shown by the third examination.

Third Examination.—Feb. 26, 1915. The pulse rate was considerably greater than at the last examination, and the superficial veins of the hands were distinctly better filled. The veins of the right hand were fuller than those of the left, and the resistance to compression of the veins by the finger was greater in the right hand. The pulse was felt in the right brachial although not strongly, and was not felt in the left brachial. Feet in bath at 2.11 p.m., in calorimeters at 2.25½, out of calorimeters at 2.49. Pulse 102, counted in the carotid, as it was difficult to count it at the right wrist, although regular.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.24	31.92	31.77		2.39	31.855	32.74	23.7
2.27	31.91	31.86	23.4	2.41	31.86	32.87	23.3
2.29	31.89	31.995	23.4	2.43	31.85	32.99	23.1
2.31	31.88	32.14	23.2	2.45	31.845	33.12	23.1
2.33	31.87	32.30	23.5	2.47	31.84	33.24	23.4
2.35	31.865	32.47	24.1	2.49	31.79	33.27	
2.37	31.86	32.61	24.1	3.18	31.42	32.79	

Cooling of foot calorimeters in 31 minutes, right 0.37°, left 0.48°. Volume of right foot 1,132 cc., of left 1,199 cc. Water equivalent of foot calorimeters with contents, right 3,812, left 3,862.

Hands in bath at 3.13½ p.m., in calorimeters at 3.23½, and out of calorimeters at 3.36.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.23	31.48	31.34	23.5	3.31	31.80	31.46	24.3
3.25	31.48	31.36	23.3	3.32	31.87	31.48	24.2
3.26	31.51	31.37	23.1	3.33	31.90	31.49	
3.27	31.58	31.385	23.4	3.34	31.95	31.505	24.3
3.28	31.62	31.40		3.35	32.00	31.52	24.2
3.29	31.70	31.42	24.1	3.36	32.12	31.53	
3.30	31.73	31.43	24.3	3.52	31.96	31.37	

Cooling of hand calorimeters, 0.16° for right and left in 16 minutes. Volume of right hand 497 cc., of left hand 457 cc. Water equivalent of calorimeters with contents, right 3,492, left 3,460. Rectal temperature 37.85°.

The blood flow in the right hand on Feb. 26 was 9.98 gm., and in the left hand 3.70 gm. per 100 cc. per minute for the last 10 minutes in the calorimeters, with average room temperature 24° C. The flow in both hands is considerably greater than two days previously, corresponding to the increased pulse rate, but the ratio is exactly the same (1 : 2.70). The flow in the right foot was only 0.70 gm. per 100 cc. per minute even with the increase in the general circulation, a considerably smaller flow than at the first examination, four months previously, while the flow in the left foot was 6.50 gm., with room temperature 23.5° C., a great increase. The ratio between the flows in the two feet was 1 : 9.28, which shows a decided decrease in the circulation in the right foot. This agrees completely with the other signs of deterioration, and suggests the probability of impending gangrene. The flow, actually measured in the right foot, is probably still sufficient to nourish the tissues, since considerably smaller flows have not infrequently been met with in other conditions without gangrene. But should a further examination show that the diminution in the flow is still progressing, that of itself would be sufficient to indicate a grave prognosis. It is an interesting fact that the ratio of the sum of the foot flows to the sum of the hand flows (1 : 1.90) is precisely the same as four months ago, in spite of the great changes in the ratios between the two hand flows and between the two foot flows, and in the absolute amount of both foot and hand flows. This suggests that the blocking of the vascular path to one leg (doubtless the diminution in the flow extends to the whole of the right posterior extremity) is associated with a reciprocal dilatation of the path to the other leg, so that the normal partition of the blood between the legs and the rest of the body is scarcely disturbed. That is to say, the blood which normally finds its way through the two common iliacs seems eventually, when the main part of the path from one common iliac is blocked, still to find its way through the one which remains pervious, the normal limb making room, it may be supposed by vasodilatation, for an additional quantity of blood.

It would, of course, be rash to generalize from one or two measurements of this kind, but the result in this case is so precise that it needs an explanation. If we reflect that one important function of the circulation, and relatively more important in the limbs than elsewhere on account of the greater proportion of their surface to their mass, is the elimination of heat, it will not appear fantastic to suggest that when the blood flow through the skin of one leg is greatly interfered with, it might be advantageous for the flow through the skin of the other leg to be accelerated. The same consideration applies to the muscles in so far as interchange between them and the other tissues is of general utility in the metabolism. The regulation of the blood pressure may also be facilitated by such a redistribution of the blood, which, if it occurs, may be assumed to be brought about, mainly at any rate, through the vasomotor system. An occlusion coming on gradually and never becoming complete presents, of course, different conditions from a complete occlusion caused by ligation of large arteries or by amputation. One important difference is that the tissues fed by

the partially occluded vessels continue to contribute to the total metabolism of the body and to the heat production, and must therefore to some extent influence the blood flow in the surfaces from which heat is lost and the organs concerned in excretion.

Note Added May 7, 1915.—Another examination of the blood flow in Costa B. was made on Apr. 7. The flow in the left hand was 3.43 gm. per 100 cc. per minute, and in the right hand 13.11 gm., with room temperature 24.5° C. (ratio 1 : 3.82). In the feet the flows were 1.51 gm. and 7.43 gm. for the right and left, respectively, with room temperature 23.3° C. (ratio 1 : 4.92). The ratio of the combined foot flows (per 100 cc. per minute) to the combined hand flows was 1 : 1.85; *i. e.*, practically the same as at the previous examinations. The man died on May 4. For 4 days before death the right leg and foot had been numb and discolored by subcutaneous hemorrhages. At the necropsy the right common iliac artery was found completely occluded by an old organized thrombus firmly adherent to the artery. A similar thrombus occupied the left subclavian artery. A fresh thrombus was found in the left external iliac artery. It was freely movable. There was marked mitral stenosis with hypertrophy of the left auricle, and the valve was covered with vegetations.

An apparent instance of a reciprocal relation of the lymph flow and blood flow in a part which leads to increased blood flow when the outflow of lymph is obstructed was observed in a case already reported.[3] The case was diagnosed as Hodgkin's disease. There was great and persistent edema in both legs and nowhere else in the body. The swelling remained quite unaltered during the five weeks of the man's stay in the hospital. It coincided with a relatively good blood flow in the feet, which was interpreted as indicating that the obstruction responsible for the edema was on the lymph path rather than on the venous path. The flow in the feet was particularly large in proportion to that in the hands (ratio of combined foot flows to combined hand flows 1 : 0.96). The sum of the foot flows per 100 cc. of part per minute actually exceeded slightly the sum of the hand flows, a very rare condition in our observations. It was suggested that far from being interfered with, the blood circulation in the edematous legs was accelerated through local vasodilatation.

It is known that the exchange between the blood in the capillaries and the tissues does not consist merely in the passage of materials out of the blood, but also in the passage of materials, including water, from the tissues into the blood. The intracellular liquids and tissue lymph are naturally in relation both with the blood and with the lymphatic lymph, and their normal composition is maintained by interchange with both. Is it not probable that when the lymphatic channels are blocked, and the elimination of waste products and the regulation of the quantity of tissue lymph by way of the lymphatics interfered with, the flow in the alternative channels, the blood capillaries, may be increased, so as to increase the excretion by way of the blood? The associated vasodilatation may very well be brought about by the accumulation of one or another of the waste products.

In the next case the question was raised at the clinical examination whether the circulatory changes observed in one hand could be at-

[3] Stewart, *Arch. Int. Med.*, 1913, xii, 678.

tributed to injury or irritation of the brachial plexus or some of its constituent cords. The blood flow measurements indicated clearly that the phenomena were due to mechanical obstruction on the arterial path of the limb, and further, a point of importance in connection with the question whether operative interference was advisable, that the blood flow in the affected hand was quite sufficient to nourish the part.

Walter L., aged 25 years, height 5 feet 10 inches, weight 210 pounds, a powerfully built man, well nourished, with a good deal of subcutaneous fat, was admitted to the City Hospital on Oct. 25, 1914, suffering from a gunshot wound. His partner who was shooting rats with a small rifle at the lunch hour accidentally shot him, the bullet (caliber No. 22) entering the right side of the chest close to the sternum and lodging near the right shoulder, as was afterwards shown by the x-ray. He drove home and then came at once to the hospital. The wound bled at first with a squirt, but soon stopped and has not bled much since. The other man was 5 feet 8 inches in height and the gun was pointed directly at the front of the patient and slightly upward. The general course of the bullet when it struck the patient was slightly upward, and very slightly to his right. On admission he complained of a continual dull ache localized under the armpit, and of pain when he breathed deeply. The fingers of the right hand are numb, but he can move them perfectly well. There is a tendency for the thumb and forefinger to flex themselves.

Nov. 1. He complains that the fingers of the right hand remain flexed rather than straight, and says that he must lay his hand upon something to keep the fingers from doubling up. It appears as if the median nerve were irritated. He can now move his right shoulder a little in all directions. There is a large hematoma in the axillary region, and the ecchymosis extends downwards to the waist line and also above the deltoid. On Nov. 4 he complains of severe pain in the deltoid region. On Nov. 5 the amplitude of the right radial pulse is less than that of the left. The pulse is regular. On Nov. 6 he still complains of his hand. The index and middle fingers now seem to be extended most of the time. On Nov. 11 there seems to be no pain in the arm, and the patient is allowed to be up. The index, middle finger, and, to a less extent, the thumb are numb. Tactile sensation and pain sensation are diminished on the palmar surface of these fingers and on the dorsal surface of their two last phalanges. The skin on the palmar surface over the median nerve distribution right up to the wrist is scaly, while the ulnar area of the palmar surface is normal. The finger nails on the right hand have grown less than on the left hand. The inner portion of the thenar eminence is atrophied. The grip between the little and ring fingers is less strong than in the left hand. The inequality in the radial pulses continues. The x-ray shows the bullet in the axilla about one inch below the lower lip of the glenoid.

The question was put by the surgeon in charge of the case, whether the diminished pulse could be attributed to injury of the

brachial plexus or to pressure on it, and, in particular, whether the blood flow in the affected hand was so much diminished as to threaten gangrene and therefore to call for surgical interference.

First Examination of Blood Flow.—Walter L. Nov. 13, 1914. Hands in bath at 2.10 p.m., in calorimeters at 2.20¾. At 2.38 left hand put into water at 8.2° C. At 2.50 left hand put into water at 44°. At 3.04 right hand out of calorimeter.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Right calorimeter.	Room.	Time.	Temperature of Right calorimeter.	Room.	Notes.
	Right.	Left.								
2.20	31.06	31.04		2.39	31.44		2.57	31.725		
2.22	31.00	31.12		2.40	31.46	23.1	2.58	31.75		
2.23	31.02	31.18	23.3	2.41	31.47		2.59	31.775	23.3	
2.24	31.03	31.27	23.4	2.42	31.48	23.2	3.00	31.79		
2.25	31.05	31.35		2.43	31.49		3.01	31.82		
2.26	31.09	31.43	23.4	2.44	31.505	23.1	3.02	31.84	23.3	
2.27	31.12	31.52		2.45	31.52		3.03	31.89		
2.28	31.14	31.595		2.46	31.56	23.1	3.04	31.925	23.4	
2.29	31.16	31.67	23.4	2.47	31.595		3.19	31.715	23.3	Lt.
2.30	31.19	31.74		2.48	31.605	23.1				31.80
2.31	31.22	31.81		2.49	31.615					
2.32	31.25	31.90	23.3	2.50	31.63					
2.33	31.28	31.98		2.51	31.64	23.3				
2.34	31.31	32.06	23.3	2.52	31.65					
2.35	31.33	32.13		2.53	31.66					
2.36	31.36	32.20		2.54	31.68	23.3				
2.37	31.39	32.28	23.2	2.55	31.695					
2.38	31.42	32.345		2.56	31.71	23.3				

Cooling of calorimeters, right 0.21° in 15 minutes, left 0.545° in 41 minutes. Volume of right hand 503 cc., of left 464 cc. Water equivalent of calorimeters with contents, right 3.497, left 3.468. Rectal temperature 37.42° C.

For 15 minutes before the vasomotor reaction was tested the blood flows of the two hands came out 5.32 gm. per 100 cc. per minute for the right hand, and 14.76 gm. for the left hand, a ratio of 1 : 2.77, with room temperature 23.2°. When the left hand was immersed in cold water the flow in the right hand was diminished to 4.71 gm. per 100 cc. per minute for the first two minutes of the immersion, to 3.31 gm. for the next 3 minutes, and it rose only to 4.83 gm. per 100 cc. per minute during the remaining 7 minutes of the period. The vasomotor reflex elicited in the right hand by immersion of the left hand in cold water was accordingly small. When the left hand was immersed in warm water the flow in the right hand sank to 3.46 gm. per 100 cc. per minute for the first 3 minutes of the period. For the next 4 minutes it rose slightly (to 4.42 gm.), for the next 3 minutes to 5.33 gm., and for the remaining 4 minutes of the period to 7.32 gm. per 100 cc. per minute.

It is clear that the initial small flow in the right hand is not due to a vasoconstriction which can either be much increased by immersion

of the contralateral hand in cold water, or much diminished by its immersion in warm water. Even the maximum flow obtained under the influence of the reflex vasodilatation is scarcely half the normal flow in the left hand. Such vasomotor reflex reactions are among the criteria of a circulation diminished by a mechanical block. In the present instance the mechanical block is not extreme, as shown by the ratio of the flows, and therefore reflex vasomotor effects on the flow are still obtainable, although diminished.

There is in any event no probability that irritation of vasoconstrictors by pressure would cause such a great and permanent discrepancy between the blood flows in the two hands. The conclusion was therefore drawn that the deficiency in the circulation of the right hand was due to pressure on the blood supply of the right arm either by the bullet itself, or by the hematoma, or by both. As regards the question whether the flow in the right hand was dangerously small, it could be answered that it was not, and that although, of course, this matter should be tested from time to time, there was no reason to apprehend gangrene with a blood flow of this magnitude.

Five days later another observation was made on the blood flow with the view of determining whether the collateral circulation was increasing. At the time of the second examination the right hand was in much the same condition as at the first examination, only the patient had observed that the finger nails on the right hand were now beginning to grow although not so fast as those on the left hand.

Second Examination.—Pulse 92. Hands in bath at 2.07 p.m., in calorimeters at 2.16½, and out of calorimeters at 2.33.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.16	31.17	31.17		2.26	31.225	31.68	
2.18	31.13	31.22	23.7	2.27	31.24	31.73	22.9
2.19	31.13	31.285		2.28	31.25	31.78	
2.20	31.13	31.34	23.3	2.29	31.27	31.82	
2.21	31.17	31.41		2.30	31.28	31.87	
2.22	31.19	31.48		2.31	31.29	31.91	23.0
2.23	31.20	31.54	23.2	2.32	31.295	31.95	23.1
2.24	31.21	31.595		2.33	31.33	32.00	
2.25	31.22	31.63	23.05	2.42	31.22	31.86	

Cooling of calorimeters in 9 minutes, right 0.11°, left 0.14°. Volume of hands, right 492 cc., left 466 cc. Rectal temperature 37.22°. Water equivalent of calorimeters with contents, right 3.489, left 3.467.

The blood flows in both hands at this examination were somewhat less than on the previous occasion (3.93 gm. per 100 cc. per minute for the right hand, and 10.76 gm. for the left). The ratio, however, between the flows in the two hands was unchanged (1 : 2.74). The room temperature (23° C.) was almost the same as at the first examination. The fact that the ratio of the flows is practically identical with that obtained at the first examination of itself almost precludes the idea that the deficiency in the right hand is due to persistent irritation of vasoconstrictors. A stimulation of this kind could hardly remain constant for 5 days, while a mechanical block might well do so.

The patient was discharged from the hospital soon afterwards.

The blood flow in his hands was again examined on Mar. 8, 1915. Since Nov. 28, 1914, he has been at work and came to the hospital merely for the examination. He says that when he first resumed work, whenever he flexed the forearm on the elbow it was done with a jerk. He finds that the right hand is still weaker than the left and gets tired sooner. There is no pain in it and he uses it freely in his work. He has some little difficulty with the right hand in such movements as those concerned in buttoning his clothes. The right arm is not quite as strong as the left. There is now no pain at the shoulder and no trace of the injury can be detected except a small scar on the right side of the chest at the point of entrance of the bullet, which still remains in the body, no attempt having been made to extract it. The left radial pulse is distinctly stronger than the right. It was not possible to feel the right brachial artery, while the left brachial pulse was strong.

Third Examination.—Hands in bath at 2.50 p.m., in calorimeters at 3.00, and out of calorimeters at 3.15. Pulse 84.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.59	32.03	32.10		3.09	32.26	32.59	23.9
3.01	32.01	32.15		3.10	32.29	32.64	23.9
3.02	32.03	32.20		3.11	32.32	32.695	
3.03	32.04	32.26	23.5	3.12	32.37	32.745	
3.04	32.06	32.31		3.13	32.41	32.795	
3.05	32.10	32.36	24.4	3.14	32.45	32.84	23.8
3.06	32.13	32.42		3.15	32.53	32.88	
3.07	32.18	32.48	24.5	3.33	32.35	32.68	
3.08	32.21	32.53	24.0				

Cooling of calorimeters in 18 minutes, right 0.18°, left 0.20°. Volume of right hand 481 cc., of left 493 cc. Rectal temperature 37.35° C. Water equivalent of calorimeters with contents, right 3.480, left 3.480.

The blood flow in the right hand at this last examination, 110 days after the previous one, was 9.30 gm. per 100 cc. per minute, and in the left hand 11.60

gm., with an average room temperature of 24° C. The sum of the flows in the two hands is only slightly greater than on Nov. 13, 1914 (20.9 gm. as against 20.08 gm.), but the distribution of the blood between the two hands is now very different, the ratio being only 1 : 1.24. Plainly a great improvement has occurred in the interval in the circulation of the right hand.

The volume measurement indicates a considerable increase in the left hand, probably due to work hypertrophy, as he still must use the left more than the right. There is a smaller diminution in the volume of the right hand due, it is to be supposed, either to absorption of a small amount of edema fluid or to slight atrophy. No edema of the hand was observed at any time, but there might have been some increase in tissue liquid not revealed by detectable swelling. The mechanical obstruction on the arterial path which was responsible for the diminished blood flow might, of course, have in some degree involved the venous outflow from the limb as well, although not to such an extent as to cause evident edema. That some pressure was exerted on nerves is indicated by the spasmodic contractions described, but there is no evidence of serious injury to nerves. The slight atrophy may well have been due simply to disuse.

Decided inequalities in the blood flow in the two hands (or feet) not associated with obvious functional or anatomical differences and not conforming to the criteria of inequalities due to a mechanical cause are sometimes observed in clinical cases, particularly, in my experience, in neuropathological conditions. The great characteristic of these inequalities of flow is their instability. Not only does the ratio of the flow in the two hands vary widely in examinations on successive days, without any apparent clinical change to account for the variation, but the ratio may one day be in favor of one hand and the next day in favor of the other, or the two flows, greatly different in amount at one examination, may be found equal at the next. A change in the external conditions (such as a sufficient increase or diminution of the air temperature) which are known to affect the vasomotor system is especially apt to alter or reverse the ratio.

For this reason it is suggested that some vasomotor peculiarity is responsible for the circulatory changes in these cases, and inequalities in the blood flow which possess the criterion mentioned are interpreted as depending on vasomotor rather than mechanical conditions. The results on a typical case (Thomas Q.) will be given.

The patient, a man 36 years of age, when he first came under observation was suffering from alcoholic neuritis affecting particularly the feet. There was no recognizable clinical difference between the two sides. Yet notable differences in the blood flow were made out, which, however, were not stable from

day to day and were therefore interpreted as depending upon vasomotor differences on the two sides not so easily abolished or perhaps more easily produced than in normal persons by the preliminary bath and the subsequent long immersion in the calorimeters. About a year after the first series of examinations the patient was again seen in the hospital, this time suffering from (tubercular) pleurisy with febrile temperature in addition to the neuritis. The change in the clinical picture was very decided and so was the change in the results of blood flow measurements. The marked tendency to cutaneous vasoconstriction already found associated with fever[4] was reflected in a greatly diminished hand and foot flow.

Thomas Q., a railroad clerk, aged 36 years, was admitted to the City Hospital on May 24, 1912, suffering from chronic alcoholism, with alcoholic neuritis. He has been drinking for his entire life. Two years ago he had "alcoholic paralysis" and collapsed while he was walking along the street after being at a dance. His limbs gave way but he did not lose consciousness and resumed his work after four days. His legs swelled and became painful, so he stopped work and went to a hospital. For the past year he has had painful micturition, especially after a drinking bout. In 18 months his weight has declined from 204 to 147 pounds, and his strength has diminished. He has had night sweats at intervals and his appetite has become poor. He has been drinking heavily since March. The legs and feet are equally untrustworthy in walking. He feels no difference between the right and left. The feet feel rather cold to him and recently there has been a tendency for chilblains to form on them. He is nervous. There is tremor of the tongue and lips. The pupils react to light and accommodation. The knee jerk is absent on both sides. There is no special defect of sensation. There is pain on pressure over the nerve trunks of both lower extremities. The upper extremities show nothing special.

On May 29, 1912, the flow in his feet was measured. It came out 1.43 gm. per 100 cc. per minute for the right, and 2.44 gm. for the left (ratio 1 : 1.70), with the rather low room temperature of 21.5°. On May 31 the flow in the right foot was 3.39 gm. per 100 cc. per minute, and in the left foot 3.87 gm. (ratio 1 : 1.14), with room temperature 25.4°. These flows are of the normal order of magnitude. Slight and transient reflex diminution of the flow in the left foot was caused by immersion of the right foot in cold water and a marked temporary diminution when the right foot was transferred to warm water. The subsequent increase of flow in the left foot, while the right continued in the warm water, only carried the flow slightly above the initial level. The much smaller flows in the feet on May 29 were probably due to the increased sensitiveness of these cases to vasoconstrictor stimulation occasioned by the considerably lower room temperature, for which there is other evidence. With the increased vasodilatation in both feet on May 31 the difference between them, if of vasomotor origin, would tend to become less.

Two examinations of the hands were made at this time. On June 4, with room temperature 25° C., the flow in the right hand was 8.51 gm. per 100 cc. per minute, in the left 13.20 gm. (ratio 1 : 1.55). The hands were not noticeably affected by neuritis at this time, but later on he returned to the hospital

[4] Stewart, *Jour. Exper. Med.*, 1913, xviii, 372.

with wrist drop in addition to foot drop. The difference in the flow in the two hands on subsequent examination was not found to be permanent, and it was therefore interpreted as due to a functional difference in vasomotor innervation. Immersion of the right hand in warm water caused, after a very slight and transient diminution in the flow (to 9.82 gm. for one minute), one of the greatest reflex increases witnessed in the whole series of observations; *viz.*, to 17.08 gm. per 100 cc. per minute. Immersion of the right hand in cold water was accompanied by a transient diminution in flow in the left hand (to 5.58 gm. per 100 cc. per minute for the first 3 minutes), the flow then increasing to 10.22 gm. per 100 cc. per minute for the remaining 7 minutes, during which the right hand continued in the cold water.

First Examination of Blood Flow.—Thomas Q. Examination of blood flow in the feet, May 31, 1912. Pulse 108. Feet in bath at 2.40½ p.m., in calorimeters at 2.55¼. 3,740 cc. of water in each calorimeter. At 3.13 p.m. the right foot was immersed in cold water at 9° C. He felt it very cold and complained much.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Left calorimeter.	Room.	Notes.
	Right.	Left.					
2.54	31.42	31.40		3.22	32.25		
2.57	31.43	31.42	25.3	3.23	32.285	25.5	
2.59	31.50	31.50	25.3	3.24	32.31		
3.01	31.56	31.56		3.25	32.35		
3.03	31.61	31.63	25.3	3.26	32.40		At 3.26 rt. foot put in water at 43° C.
3.05	31.675	31.70		3.27	32.41		
3.06	31.72	31.75		3.28	32.425		
3.07	31.75	31.79	25.4	3.29	32.45		
3.09	31.805	31.86		3.30	32.49	25.7	
3.11	31.88	31.93	25.45	3.31	32.525		
3.13	31.93	32.00		3.32	32.525		Stirring was brief and insufficient.
3.15		32.07		3.33	32.58		
3.17		32.11	25.5	3.34	32.60		Stirring insufficient.
3.18		32.14		3.35	32.635		Foot out of calorimeter.
3.19		32.165		3.44	32.525		Rt. is now at 31.595° C.
3.20		32.21					
3.21		32.23					

Cooling of calorimeters, right 0.335 in 31 minutes, left 0.11 in 9 minutes. Volume of right foot in calorimeter 1,311 cc., of left foot 1,290 cc. Water equivalent of calorimeters with contents, right 4,857, left 4,843. Rectal temperature 37.35°.

Second Examination.—Examination of flow in hands, June 4, 1912. Hands in bath at 2.23 p.m., in calorimeters at 2.33. At 2.47 p.m. the right hand was put into water at 8° C.; at 2.57 into water at 43°. At 3.07 p.m. the left hand was taken out of the calorimeter.

Cooling of calorimeters, right 0.33° in 39 minutes, left 0.13° in 9½ minutes. Volume of right hand 460 cc., of left hand 437 cc. Water equivalent of calorimeters with contents, right 3,463, left 3,445. Pulse 100. Rectal temperature 37.5.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Time.	Temperature of Left calorimeter.	Room.	Notes.
2.31½	31.57	31.51	24.8	2.52	32.53	24.95	
2.34	31.57	31.53		2.53	32.57		
2.35	31.63	31.62	25.2	2.54	32.605		
2.36	31.695	31.72		2.55	32.635		
2.37	31.74	31.78	25.0	2.56	32.66		
2.38	31.79	31.86		2.57	32.71		
2.39	31.83	31.92		2.58	32.745	25.1	
2.40	31.87	31.96		2.59	32.805		
2.41	31.895	32.02		3.00	32.86		
2.42	31.94	32.08		3.01	32.925	25.1	
2.43	31.995	32.15	25.0	3.02	32.995		
2.44	32.03	32.225		3.03	33.07		
2.45	32.08	32.29		3.04	33.135	25.15	
2.46	32.11	32.34		3.05	33.19		
2.47	32.14	32.38		3.06	33.25		
2.48		32.40		3.07	33.32	25.2	
2.49		32.41		3.16	33.19		Rt. 31.81
2.50		32.43					
2.51		32.48					

That the cause of the inequality between the flows in the two hands observed on June 4, 1912, was a transient functional difference is indicated by the result of the third examination on the following day (June 5). Here with a lower room temperature (22.4°) the flow was reduced in both hands, but far more in proportion in the left (7.31 gm. per 100 cc. per minute for the right, 7.35 gm. for the left hand). The excess in the flow in the left hand is, however, in reality somewhat greater than the numerical results indicate. For the left hand had suffered the loss of a portion of the thumb by amputation a while ago, and the ratio between its surface and its mass was therefore diminished. Also the man is right handed and in right handed persons the flow per 100 cc. of volume is usually somewhat greater than the left.

Third Examination.—June 5, 1912. He says he is feeling well and can walk fairly, although he soon gets tired. He was out on the hospital grounds yesterday and today. Hands in bath at 1.22½ p.m., in calorimeters at 1.32, out of calorimeters at 1.50. Pulse 110.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Time.	Temperature of Calorimeters. Right.	Left.	Room.
1.31½	31.08	31.05		1.42	31.495	31.39	
1.33	31.07	31.03	22.2	1.43	31.52	31.415	22.4
1.34	31.11	31.07		1.44	31.575	31.45	
1.35	31.175	31.12	22.3	1.45	31.61	31.49	22.4
1.36	31.21	31.15		1.46	31.64	31.52	
1.37	31.27	31.20	22.4	1.47	31.69	31.55	
1.38	31.31	31.24		1.48	31.72	31.59	22.4
1.39	31.39	31.30		1.49	31.76	31.625	
1.40	31.425	31.335		1.50	31.785	31.635	
1.41	31.47	31.36		1.58	31.665	31.525	

Cooling of calorimeters in 8 minutes, right 0.12°, left 0.11°. Volume of right hand 468 cc., of left hand 436 cc. Water equivalent of calorimeters with contents, right 3,469, left 3,444. Rectal temperature 37.7°.

Thomas Q. was discharged from the hospital on June 29, 1912, and was readmitted on Jan. 19, 1913. He has been drinking for 4 weeks and is decidedly worse than at the previous admission. He cannot walk at all and has wrist drop of both hands. He first noticed the wrist drop the day he came. In both extremities the extensor muscles are more involved than the flexors. The nerve trunks are tender, the reflexes absent. He was treated by hot baths and massage of the extensor muscles. He states that if he is made to sit around even a few minutes after the bath he gets a severe chill lasting 20 to 30 minutes, which does not occur if he is put straight to bed. He never has a chill except after the hot bath. This is of interest as it corresponds with the evidence of increased susceptibility to reflex vasoconstriction of the cutaneous blood vessels deduced from the blood flow examinations. The flow in the hands was examined on May 21 and again on May 24, 1913, and the flow in the feet on May 24. At this time he had some fever and on May 29 the physical signs of pleurisy were found. On June 15 and again on June 18, 1913, tubercle bacilli were found in the sputum, and he was sent to the sanitarium on June 24.

When examined on May 21, 1913, nearly a year after the first examination, the blood flow in the right hand was found to be only 4.49 gm. per 100 cc. of hand per minute, and in the left hand 3.06 gm. (ratio 1 : 1.46), with the relatively high room temperature of 26.4° C. These flows were for the last 12 minutes in the calorimeters. His rectal temperature was much above the normal (40.45° C.), and the tendency to vasoconstriction associated with fever is no doubt a factor in the small flow. This tendency is illustrated by the tardiness with which the rate at which the thermometers rose became steady. Three days later (on May 24, 1913) the flow in the hands was still smaller (2.31 gm. per 100 cc. per minute for the right hand and 2.26 gm. for the left hand, for 12 minutes in the calorimeters before the testing of the vasomotor reaction, with room temperature 23.2° C.). Calculated for the last 7 minutes of this period the flows were only 1.71 and 1.58 gm. per 100 cc. per minute for the right and left hands respectively, denoting a great tendency to the onset of vasoconstriction. A rapidly increasing vasoconstriction revealed by a decrease in the rate of heat loss to the calorimeters has been observed in other cases of fever; for instance, in pneumonia before the rapid rise of body temperature. In one case in which the condition had not as yet revealed itself clearly by the physical signs, this behavior of the hands in the calorimeters suggested the onset of fever, the temperature rose nearly 5° F. within eight hours, and the next day physical signs of pneumonia were present.

During immersion of the right hand in warm water, the flow in the left, far from being increased, was actually diminished (to 1.31 gm. per 100 cc. per minute), a further illustration of the increasing vasoconstriction, which cannot be overcome by the reflex vasodilatation normally associated with immersion of the contralateral hand in warm water. The patient felt worse than at the last examination.

The flow in the feet was also abnormally small (0.84 gm. per 100 cc. per

minute for the right foot, and 0.87 gm. for the left for a period of 20 minutes in the calorimeters, with room temperature 23.3° C.). For the last 10 minutes of this period the flows were only 0.77 and 0.72 gm. per 100 cc. per minute for the right and left foot, respectively, showing that the vasoconstriction was increasing in the feet also with the duration of immersion. Naturally with a tendency to vasoconstriction manifested so strongly in both hands and feet, the ratio of foot to hand flow (1:2.6) lies within the normal limits, whereas with a lesser degree or less general distribution of the vasoconstriction this ratio is apt to be markedly disturbed in favor of the hand flow.

Cases do occur, although in my experience quite rarely, in which without any obvious reason a marked inequality in the flow in the two hands is present and persists indefinitely, without reversal of the ratio. The ratio, however, is unstable, varying greatly from day to day, and this differentiates the condition from inequalities of mechanical origin and places it in the vasomotor group. This condition is illustrated in the case of William F.

William F., a laborer, aged 50 years, was admitted to the City Hospital on March 22, 1912, suffering from "combined system disease." His illness began last June with difficulty in walking, and this has become rapidly worse. Sometimes he almost falls. About three months before admission he had a severe nose-bleed lasting for three nights without known cause. For the past year he has suffered from severe frontal headache lasting from six to twenty-four hours. He also complains of pain in the pit of the stomach coming on after eating. No vomiting. He sweats much at night, and the sweats are followed by chills. The sweating is so copious that his gown is soaked in five minutes. He says he used to be "hot-blooded," wearing no overcoat in winter, but now he is always chilly. His feet get cold as ice in bed. His hands also get cold now, although not so readily as the feet. When he goes to bed at night his feet are apt to be swollen, but the swelling is all gone in the morning. He had chancre twenty years ago and it was not treated. He is married but never had any children. His wife had two or three miscarriages. He does not drink or chew tobacco. He walks with a spastic gait. Knee jerks exaggerated, and ankle clonus can be elicited on both sides. There is some toe drop on the left side. He says he has pain all the time in his "bones," in the legs and back, but not much pain in the arms. There seems to be some loss of sensation in the left leg, and he cannot always tell the difference between warmth and cold there. Pain perception is prompt, and vibration sense good. His power of localization is somewhat impaired. He can stand with his eyes closed. Both pupils react normally to light. No obvious wasting of the hands. The grip of both hands is fairly good, the right somewhat stronger than the left. He can not write now, although a well educated man. Blood count on Mar. 24, 1912, erythrocytes 5,480,000, leucocytes 7,400. Mar. 29, Wassermann test negative. Apr. 17, spinal fluid clear. Cell count 6 per cc. X-ray examination negative. Urine, nothing special. Heart examination negative. The blood flow in the

hands was examined on Apr. 24, 1912, and that in the hands and feet the following day (Apr. 25).

First Examination of Blood Flow.—William F. Apr. 24, 1912. Hands in bath at 2.43 p.m., in calorimeters at 2.52. At 3.15 p.m. left hand put into water at 44° C. Pulse 100. Mouth temperature 36.65°. At 3.29 left hand put in water at 8° C. At 3.41 left hand put in water at 43° C. At 3.48 hand out of calorimeter.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Right calorimeter.	Room.	Time.	Temperature of Right calorimeter.	Room.	Notes.
	Right.	Left.								
2.51	30.52	30.55		3.16	30.79	23.5	3.38	31.46		
2.53	30.47	30.50	22.3	3.17	30.80		3.39	31.49		
2.54	30.47	30.49		3.18	30.81		3.40	31.50		
2.55	30.47	30.49		3.19	30.84		3.41	31.505		
2.56	30.46	30.48		3.20	30.88		3.42	31.52		
2.57	30.46	30.47		3.21	30.915		3.43	31.525		
2.59	30.45	30.46		3.22	30.975	22.2	3.44	31.53	22.25	
3.00	30.45	30.455		3.23	31.02		3.45	31.54		
3.01	30.455	30.455	22.5	3.24	31.08		3.46	31.55		
3.03	30.48	30.45		3.25	31.105		3.47	31.56		
3.04	30.50	30.44		3.26	31.15		3.48	31.57		
3.05	30.52	30.435		3.27	31.195	22.0	3.56½	31.46		Lt. 30.04
3.06	30.54	30.445	22.5	3.28	31.24					
3.07	30.58	30.44		3.29	31.295					
3.08	30.60	40.44		3.30	31.305					
3.09	30.63	30.435		3.31	31.32					
3.10	30.67	30.435		3.32	31.335					
3.11	30.695	30.445		3.33	31.35	22.0				
3.12	30.705	30.445		3.34	31.365					
3.13	30.73	30.45		3.35	31.38					
3.14	30.75	30.455		3.36	31.40					
3.15	30.78	30.45		3.37	31.43					

Cooling of calorimeters, right 0.11° in 8½ minutes, left 0.41° in 41½ minutes. Volume of right hand, 517 cc., of left hand 523 cc. Water equivalent of calorimeters with contents, right 3,509, left 3,513. Rectal temperature 37.4° C.

On Apr. 24 the flow in the right hand came out 4.20 gm. per 100 cc. per minute; in the left, 1.11 gm. (ratio 1 : 3.78), with room temperature 22.5°, an average flow much below the normal. Immersion of the left hand in warm water caused a good contralateral vasomotor reflex, indeed considering the long duration of the vasodilatation, an exaggerated one. The vasodilatation was preceded in the normal way by a good vasoconstriction for the first 3 minutes of immersion of the left hand. The diminution of the flow in the right hand on immersion of the left in cold water was also prompt, substantial, and durable.

The next day the flow in the right hand was 5.83 gm., in the left 3.11 gm. (ratio 1 : 1.87), with room temperature 23.5°, still a marked preponderance in favor of the right hand.

In the feet the flow was remarkably small but the relative preponderance in the right foot was precisely the same as in the right hand, the ratio of the flow

in the right hand to that in the right foot being 15:1, and the corresponding ratio for the left hand and foot also 15:1. Immersion of the left foot in warm water caused, if anything, a diminution of the flow in the right foot, but the change was insignificant.

The patient was discharged from the hospital at his own request on May 12, and readmitted on May 31, 1913. On June 6, 1912, the blood examination showed hemoglobin 85 per cent., leucocytes 11,800. He says his legs now get red if rubbed or scratched and feel "burning." This was not the case when he was in the hospital before. He describes the heat as coming "from the inside of the legs, from the bone." Neither the legs nor the feet feel warm to the observer's hand. The veins of the legs are larger than before. He does not sweat much now. The knee jerk is still exaggerated, and ankle clonus can be elicited. He is less able to walk than when previously in the hospital and must use a stick, which was not the case before. Romberg's sign is not present. He has had much trouble in urination for over a year, with burning pain in the penis. In the sitting position he can easily make water, but not standing up. The pulse in the left brachial is distinctly smaller than in the right. This is easier to make out than any difference between the two radials. He was discharged from the hospital "unimproved," Oct. 6, 1912. The blood flow in the hands was examined on June 11, and that in the feet on June 12, 1912.

Second Examination.—June 11, 1912. Hands in bath at 2.16 p.m., in calorimeters at 2.26¼, and out of calorimeters at 2.50.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Time.	Temperature of Calorimeters. Right.	Left.	Room.
2.25	31.50	31.40	24.5	2.39	31.65	31.43	
2.27	31.47	31.36		2.40	31.665	31.43	24.7
2.28	31.48	31.355	24.5	2.41	31.68	31.435	
2.29	31.49	31.355		2.42	31.695	31.44	
2.30	31.50	31.36	24.6	2.43	31.715	31.45	
2.31	31.515	31.365		2.44	31.73	31.455	
2.32	31.535	31.375		2.45	31.755	31.465	24.8
2.33	31.56	31.395		2.46	31.78	31.49	
2.34	31.595	31.40	24.8	2.47	31.80	31.51	
2.35	31.60	31.41		2.48	31.815	31.52	24.7
2.36	31.61	31.42		2.49	31.83	31.525	
2.37	31.625	31.425	24.7	2.50	31.85	31.53	
2.38	31.64	31.425		3.04	31.69	31.39	

Cooling of calorimeters in 14 minutes, right 0.16°, left 0.14°. Volume of right hand 493 cc., of left 499 cc. Water equivalent of calorimeters with contents, right 3,489, left 3,494. Rectal temperature 37.6°.

On June 11 the flow in the right hand was 4.40 gm., in the left 2.77 gm. (ratio 1:1.58) with room temperature 24.7° C. On June 12 the flows were 0.82 and 0.48 gm. in the right and left foot, respectively. Immersion of the right foot in warm water caused practically no change in the left foot. The foot flows are still abnormally small, although larger than on Apr. 25. It is to be remarked,

however, that the ratio between the flow in the right hand and that in the right foot (5.4:1) is again almost the same as the ratio between the flow in the left hand and that in the left foot (5.3:1). In other words, the relative preponderance of flow is almost the same in the right foot as in the right hand. The blood flow in the right hand and foot in this patient is therefore, it would appear, permanently and decidedly greater than in the left hand and foot, although at the time of examination no marked clinical difference in the condition of the two sides of the body could be detected. The variability in the ratio between the flows in the two hands from time to time, in the absence of material variations in the factors which determine general vasomotor changes (*e. g.*, decided changes in the external temperature), differentiates the condition from an inequality due to a mechanical cause and stamps it apparently as a functional peculiarity.

The curious fact that when the ratio between the flows in the two hands varies, the ratio between the flows in the two feet varies to the same amount, would seem to require for its explanation the assumption that the intensity of the vasomotor innervation of one-half of the body, or at least of the extremities on one side, is simultaneously affected and to the same degree with respect to the innervation of the other half. Changes in the bulb, for example in its circulation, affecting the "general vasomotor center," not necessarily exclusively on one side, but to a greater degree on one side than on the other, might be expected to produce such an effect. If the change responsible for the phenomenon were located below this level it would seem that the whole dorsolumbar region from which the vasoconstrictor outflow takes place would require to be subjected to it. Is there here, perhaps, an indication of a somewhat greater progress of the pathological change on one side than on the other, a difference which has not as yet otherwise revealed itself? If the primary lesion in this case is in the upper motor neurone, it is not unnatural to suppose that the vasomotor reflex arcs may maintain an even increased vasoconstrictor tone associated with a relatively small peripheral blood flow corresponding to the exaggeration of the skeletal reflexes and the spasticity of the skeletal muscles.

A marked variability in the ratio of the blood flows in the two hands (or feet) in our observations on normal persons has not been seen. But in one normal case a decided permanent difference in the flows in the two hands was made out, the ratio of the flows remaining practically constant in observations made at an interval of 3 days and varying surprisingly little even over long periods.

In John R., a normal man, at that time 20 years old, the flow on Mar. 22, 1913, in the right hand was 10.08 gm., and in the left 7.25 gm. per 100 cc. per minute, with room temperature 24.0° C. for a period of 13 minutes, the ratio of the flows being 1:1.39. On Mar. 25, 1913, the flows were 12.38 and 8.82 gm., respectively, for the right and left hands (ratio 1:1.40) with room temperature 24.8° C. for a period of 15 minutes. On Nov. 14, 1914, the flows were 14.85 gm. for the right hand and 10.07 gm. per 100 cc. per minute for the left hand

for a period of 22 minutes, with room temperature 24.3° C. (ratio 1 : 1.47). There is no anatomical or functional difference between the two hands or arms, nor any history of injury which would explain such a difference in the blood flow. The stability of the ratio is strongly in favor of a mechanical explanation, a congenital difference in the cross-section of the two subclavians, for example, rather than an explanation based on a difference on the two sides in the permanent vasomotor tone or a difference in the reflex vasomotor reaction to the manipulations and external conditions connected with the measurements. No such difference was found in the foot flows, the ratio being 1 : 1.05 in the supine and 1 : 1.13 in the sitting position on Mar. 25, 1913, with room temperatures 25.1° and 24.6°, respectively. It was at first supposed that some as yet latent unilateral pathological process, for instance pulmonary tuberculosis, might be connected with the anomaly, but nothing has developed to justify that suggestion. The subject stated at the time of the last examination that the tendency to free bleeding from slight injuries and especially from the nose, from which he had suffered for 15 years, has now disappeared.[5]

John R. Nov. 14, 1914. Hands in bath at 11.42 a.m., in calorimeters at 11.55, out of calorimeters at 12.08 p.m.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
11.54	32.12	32.22	24.1	12.08	32.67	32.51	
11.56	32.12	32.22		12.09	32.70	32.53	
11.57	32.16	32.24	24.2	12.10	32.75	32.555	24.3
11.58	32.20	32.255		12.11	32.79	32.58	
11.59	32.23	32.27		12.12	32.82	32.62	24.3
12.00	32.285	32.305	24.3	12.13	32.88	32.64	
12.01	32.32	32.32		12.14	32.91	32.66	
12.02	32.38	32.35		12.15	32.94	32.67	24.4
12.03	32.42	32.37		12.16	32.97	32.68	
12.04	32.48	32.40	24.3	12.17	32.995	32.69	
12.05	32.52	32.43		12.18	33.02	32.695	24.3
12.06	32.56	32.45		12.28	32.88	32.555	
12.07	32.61	32.48	24.3				

Cooling of calorimeters, right and left 0.14°, in 10 minutes. Volume of right hand 387 cc., of left hand 357 cc. Water equivalent of calorimeters with contents, right 3.405, left 3.381. Rectal temperature 36.64° C. Pulse 62. Blood pressure, right arm 98 (systolic), 84 (sudden change in sound), 76 (cessation of sound). Another observation 98, 83, 74. Left arm 97, 79, 65.

Note Added May 7, 1915.—On this day, which was close and muggy, the flow in the right hand was 18.52 gm. and in the left 14.01 gm. (ratio 1 : 1.32) with room temperature 25.1° C.

As a practical point of technique it may be suggested that when it is desired to test the stability of the ratio between the flows on the

[5] Stewart, *Jour. Exper. Med.*, 1913, xviii, 354.

two sides, in cases where the question arises whether the cause is a nervous or a mechanical one, this could perhaps often be quickly done by measuring the blood flow several times on the same day with considerably different room and calorimeter temperatures. If the inequality is of vasomotor origin, it may be expected to disappear or be greatly reduced in one or more of the sets of observations, whereas an inequality due to a permanent mechanical cause could not disappear.

SUMMARY.

1. In cases in which great inequalities in the blood flow in the two hands were produced by mechanical causes (ligation or compression of vessels, embolism), the stability of the ratio of the flows, in successive measurements at short intervals, was found to be characteristic. Over long intervals the opening up of collateral circulation or the progressive increase of the block (in a case of multiple embolism with thrombosis) was followed by changes in the ratio of the blood flows in the normal and the affected part. Another criterion of these conditions was found to be that the inequality was not abolished by producing general vasomotor changes; *e. g.,* by altering the external temperature.

2. In certain cases inequalities in the blood flow in the two hands (or feet) were found which were not stable from day to day, and which could be abolished, reduced, increased, or reversed by alterations in the external conditions which bring about general vasomotor changes. These inequalities, not associated with clinically recognizable differences between the parts compared, were interpreted as due to unequal activity of the vasomotor mechanism on the two sides. The condition appeared to be most frequent in certain groups of neurological cases.

I wish to express my obligations to the staff of the City Hospital and to my colleagues at Lakeside Hospital for many courtesies.

TABLE I.

Date.	Case.	Age.	Pulse rate.	Blood pressure.	Temperature (C.) of				Volume of part in cc.		Heat given off in gm.-cal.			Blood flow in gm. per min.		Flow per 100 cc. of part per min.		Notes.
					Room.	Art'l blood.	Calorimeters. Right.	Calorimeters. Left.	Right.	Left.	Right.	Left.	In min.	Right.	Left.	Right.	Left.	
Oct. 26, 1914	Costa B...	47 yrs.	102	114.85	22.0	36.95	31.81	31.42	473	441	1980	424	15	28.53	5.67	6.03	1.28	Hands } Embolism of lt. hand
					22.0	36.85	31.68	31.99	1191	1188	1040	1946	15	14.90	29.67	1.25	2.50	Feet } and rt. leg.
Feb. 24, 1915			81	110.75	23.8	37.05	32.07	31.86	498	452	1711	690	11	34.70	11.48	6.96	2.54	Hands.
Feb. 26, 1915			102		24.0	37.35	31.82	31.45	497	457	2469	900	10	49.60	16.95	9.98	3.70	Hands.
					23.5	37.25	31.87	32.71	1132	1199	694	5847	18	7.96	77.95	0.70	6.50	Feet.
Nov. 13, 1914	Walter L..	25 yrs.			23.3	36.92	31.22	31.77	503	464	2063	4769	15	26.80	68.59	5.32	14.76	Hands. Bullet in rt. shoulder.
					23.1	.....	31.44	.....	....	....	234	....	2	23.72	.....	4.71	.....	Lt. hand in water at 8.2° C.
					23.2	.....	31.48	.....	....	....	245	....	3	16.68	.....	3.31	.....	Lt. hand still in cold water.
					23.1	.....	31.56	.....	....	....	821	....	7	24.31	.....	4.83	.....	Lt. hand still in cold water.
					23.3	.....	31.65	.....	....	....	248	....	3	17.42	.....	3.46	.....	Lt. hand in water at 44° C.
					23.3	.....	31.69	.....	....	....	420	....	4	22.26	.....	4.42	.....	Lt. hand still in warm water.
					23.3	.....	31.76	.....	....	....	374	....	3	26.84	.....	5.33	.....	Lt. hand still in warm water.
					23.3	.....	31.86	.....	....	....	671	....	4	36.83	.....	7.32	.....	Lt. hand still in warm water.
Nov. 18, 1914			92	R. 115.80	23.0	36.72	31.22	31.67	492	466	1246	2964	13	19.36	50.16	3.93	10.76	Hands.
Mar. 8, 1915				L. 130.80	24.0	36.85	32.30	32.60	481	493	2015	2407	11	44.73	57.20	9.30	11.60	Hands.
May 29, 1912	Thomas Q.	36 yrs.	84		21.5	36.50	30.67	30.80	1265	1262	1708	2846	18	18.08	30.82	1.43	2.44	Feet. Alcoholic neuritis.
May 31, 1912			108		25.4	36.75	31.68	31.71	1311	1290	3250	3630	16	44.51	50.01	3.39	3.87	Feet.
					25.5	.....	.....	31.98	....	....	....	1119	6	.....	43.44	.....	3.36	Rt. foot in water at 9° C.
					25.5	.....	.....	32.18	....	....	....	1501	7	.....	52.13	.....	4.04	Rt. foot still in cold water.
					25.7	.....	.....	32.31	....	....	....	238	2	.....	29.78	.....	2.30	Rt. foot in water at 43° C.
					25.6	.....	.....	32.43	....	....	....	1405	7	.....	51.62	.....	4.00	Rt. foot still in warm water.
June 4, 1912			100		25.0	37.00	31.86	31.95	460	437	2355	3410	13	39.16	57.71	8.51	13.20	Hands.
					25.0	.....	.....	32.40	....	....	....	393	3	.....	24.39	.....	5.58	Rt. hand in water at 8° C.
					25.0	.....	.....	32.57	....	....	....	1247	7	.....	44.68	.....	10.22	Rt. hand still in cold water.
					25.1	.....	.....	32.73	....	....	....	165	1	.....	42.93	.....	9.82	Rt. hand in water at 43° C.
					25.1	.....	.....	33.03	....	....	....	1240	9	.....	74.66	.....	17.08	Rt. hand still in warm water.
June 5, 1912			110		22.4	37.2	31.43	31.33	468	436	3330	2879	17	34.23	32.05	7.31	7.35	Hands.

TABLE I.—*Concluded.*

Date.	Case.	Age.	Pulse rate.	Blood pressure.	Temperature (C.) of				Volume of part in cc.		Heat given off in gm.-cal.			Blood flow in gm. per min.		Flow per 100 cc. of part per min.		Notes.
					Room.	Art'l blood.	Calorimeters. Right.	Calorimeters. Left.	Right.	Left.	Right.	Left.	In min.	Right.	Left.	Right.	Left.	
May 21, 1913	Thomas Q.		140		26.4	39.95	31.43	31.35	400	379	1656	1077	12	17.99	11.59	4.49	3.06	Hands. Much worse. Has fever.
May 24, 1913			116	85.60	23.2	40.1	31.24	31.20	393	372	869	810	12	9.08	8.42	2.31	2.26	Hands.
					23.5	.....	31.28	.....	....	....	409	....	10	5.15	.....	1.31	.....	Lt. hand in water at 44° C.
					23.3	40.0	30.91	30.86	1063	1057	1460	1520	20	8.92	9.23	0.84	0.87	Feet.
Apr. 24, 1912	Wm. F....	50 yrs.	100		22.5	36.9	30.62	30.45	517	523	1719	474	14	21.72	5.83	4.20	1.11	Hands. Combined degeneration.
					23.5	.....	30.80	.....	....	....	228	....	3	13.84	.....	2.67	.....	Lt. hand in water at 44° C.
					22.1	.....	31.05	.....	....	....	2193	....	11	37.86	.....	7.32	.....	Lt. hand still in warm water.
					22.0	.....	31.34	.....	....	....	561	....	6	18.68	.....	3.61	.....	Lt. hand in water at 8° C.
					22.0	.....	31.44	.....	....	....	702	....	6	23.81	.....	4.60	.....	Lt. hand still in cold water.
					22.2	.....	31.54	.....	....	....	544	....	7	16.11	.....	3.11	.....	Lt. hand in water at 43.4° C.
Apr. 25, 1912			104		23.5	36.9	30.78	30.63	493	502	2058	1188	13	28.74	15.64	5.83	3.11	Hands.
					22.4	36.8	30.42	30.35	1370	1265	742	366	24	5.38	2.62	0.39	0.21	Feet.
					22.1	.....	30.32	.....	....	....	262	....	10	4.49	.....	.....	0.32	Lt. foot in water at 43° C.
June 11, 1912					24.7	37.1	31.76	31.48	493	499	1046	699	10	21.76	13.82	4.40	2.77	Hands.
June 12, 1912			108		23.4	36.85	30.82	30.73	1360	1317	855	492	14	11.25	6.38	0.82	0.48	Feet.
					23.6	.....	.....	30.68	....	....	....	369	10	.....	6.64	.....	0.50	Rt. foot in water at 43° C.
Nov. 14, 1914	John R....	22 yrs.	62	98.75	24.3	36.14	32.57	32.46	387	357	4065	2620	22	57.50	35.95	14.85	10.07	Hands.

HIBERNATION AND THE PITUITARY BODY.

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Plates 1 to 3.

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INTRODUCTION.

Not only has the phenomenon of sleep offered a problem which has not been solved to the satisfaction of physiologists,[1] but still less satisfactory have been the explanations accounting for the prolonged dormant periods to which certain species of animals are subject, and of which the hibernation of winter latitudes and æstivation of tropical and arid regions are the most notable examples. Marshall Hall[2] expressed the opinion nearly a century ago that natural sleep, hibernation, and the so called diurnation of animals with nocturnal habits, differed from one another merely in degree, and this idea has again been revived by various writers.

According to the prevailing views of hibernation,—the form of seasonal sleep to which chief attention has been paid,—the lethargy, which is the striking feature of the condition, is attributable to two extracorporeal factors, namely, low external temperature and diminished food supply.[3] However, such attempts as have been made to determine the influence of these factors under experimental conditions have given results which are not entirely convincing.

Of late years some attention has been paid to the central nervous system and the histological alterations which occur in the nerve cells

[1] Piéron, H., Le problème physiologique du sommeil, Paris, 1913.

[2] Hall, M., On Hybernation, *Phil. Tr. Roy. Soc.*, 1832, pt. 1, 335.

[3] A résumé of the subject with bibliography will be found in Merzbacher, L., Allgemeine Physiologie des Winterschlafes, *Ergebn. d. Physiol.*, 1904, iii, pt. 2, 214.

of dormant animals,[4] but the larger number of the more recent studies upon hibernation deal with respiratory problems. These studies have shown (Nagai,[5] and others) that there is a marked decrease in the respiratory quotient during the sleeping period, while at the time of awakening, coincident with the rapid rise in body temperature, the output of carbon dioxide is greatly increased, presumably through combustion of stored glycogen and fat.[6] Valuable as these observations have been, they have not served to shed light on the underlying cause of the phenomenon of hibernation. They have merely added to our knowledge of the physiology of the state when once induced.

Though lethargy is the dominant manifestation of hibernation, it is important in our present connection to recall certain other factors of the condition, particularly the preliminary storage of fat and the subsequent evidences, during the period of somnolence, of retarded tissue combustion. One expression of this is the lowering of the body temperature—a matter which aroused the inquisitive mind of John Hunter, who, with Jenner, was perhaps the first to make accurate studies in this direction. Additional factors are the brachycardia, the slowed respiration with diminished output of carbon dioxide, the lowered blood pressure, the relative peristaltic inactivity, and the marked insensitivity to painful or emotional stimuli.

It is well known that secretory overactivity, or even the administration of extracts of certain of the glands of internal secretion, is capable of accelerating tissue metabolism with the production of symptoms the converse of those recounted above. The purpose of the present communication is to point out that a seasonal wave of physiological inactivity on the part of these glandular structures may well account for the phenomenon of hibernation.

[4] Zalla, M., Recherches expérimentales sur les modifications morphologiques des cellules nerveuses chez les animaux hibernants. (Résumé), *Arch. ital. de biol.*, 1910–11, liv, 116.

[5] Nagai, H., Der Stoffwechsel des Winterschläfers, *Ztschr. f. allg. Physiol.*, 1909, ix, 243.

[6] Pembrey, M. S., Further Observations upon the Respiratory Exchange and Temperature of Hibernating Mammals, *Jour. Physiol.*, 1903, xxix, 195.

The Somnolence of Experimental and Clinical Hypopituitarism.

Experimental Observations.—In the course of our earlier experiences with experimental hypophysectomy in the canine, it was observed[7] that after a total or nearly total surgical removal of the gland the animals, though they remained vigorous and active for a day or more, soon passed into a profoundly lethargic state, which often terminated within the next forty-eight hours in a fatal coma. In describing this state of so called cachexia hypophyseopriva, which had been previously observed by Vassale and Sacchi, by Paulesco, and some others, it was pointed out that a lowering of body temperature was one of the inaugural symptoms, and that in the fatal cases the temperature often fell to that of the surrounding medium, rectal registrations of 20° C. being not uncommon. It was noted, moreover, in the case of animals subjected to an incomplete though nearly total extirpation that a pronounced lethargy with coincident fall in body temperature was more apt to supervene in the winter months than after similar operations in warmer seasons. This we at first attributed to the fact that the animal's quarters were insufficiently heated, but we soon found that the fall in temperature was characteristic of the condition regardless of the surrounding temperature.

In addition to the persistent and often extreme lowering of body temperature, it was observed that respiration became shallow and slow, in some cases registering only three or four respirations a minute, that the pulse rate was usually retarded, that the blood pressure was very low, and that there was a peculiar insensitiveness to external painful stimuli, often so complete that the animal might not be aroused from its lethargy even by the taking of the blood pressure registration from the open femoral.

Though the main points which we wish to emphasize in connection with these experimental states of hypophysial insufficiency are the lethargy, the subnormal temperature, the slow pulse and respiration, the lowered blood pressure, and the insensitivity, following the total or nearly total deprivation of the gland, there are other matters

[7] Crowe, S. J., Cushing, H., and Homans, J., Experimental Hypophysectomy, *Bull. Johns Hopkins Hosp.*, 1910, xxi, 127.

of interest in our present connection. It was observed both by Crowe and Goetsch that the hearts of the animals which had been in a lethargic state for some hours would continue to beat, after the fashion of a batrachian heart, for an unusual length of time when removed from the body after death.[8] It was found that the subnormal body temperature could, with some difficulty, be raised approximately to the normal by the application of external heat (wrapping in electric pads, etc.),—an effect, however, which usually led to extreme panting and which appeared to accelerate rather than to postpone the fatal issue after a total hypophysectomy. It was also observed that the injection of pituitary extract was the most effective means of raising the body temperature with coincident improvement in the animal's condition; and by such injections threatened states of cachexia hypophyseopriva were seemingly forestalled in a number of animals in which at the operation a small fragment, sufficient to maintain life, had been left, though for a time it seemed to have lost its capacity to furnish the necessary secretion.

In the course of these earlier studies we made what proved to be, from a clinical standpoint, the most important of our observations; namely, that animals surviving after a considerable deprivation of the gland, though not sufficient to precipitate a fatal state of cachexia hypophyseopriva, tended to become adipose and to lose their sexual activity. This discovery furnished the first experimental explanation of the clinical syndrome of dystrophia adiposogenitalis (*typus Fröhlich*), by demonstrating that the condition was in all certainty due to hypophysial insufficiency.[9]

As a rule, these adipose animals were apt to be drowsy and apathetic, with a slowed pulse and a more or less persistently subnormal temperature, which could be raised by the administration of hypo-

[8] This we have since observed in the case of a hibernating woodchuck, which we have autopsied, cardiac pulsations having continued for half an hour.

It may be recalled in this connection that attention has been drawn by others to the resistance (*Ueberleben*) of the nervous system of the hibernating animal. Both Valentin and Schiff have shown that the nerve-muscle preparation of the hibernating marmot is almost as slow in losing its excitability as is that of the frog.

[9] Cushing, H., The Hypophysis Cerebri. Clinical Aspects of Hyperpituitarism and Hypopituitarism, *Jour. Am. Med. Assn.*, 1909, liii, 249.

physial extracts.[10] Thus, in addition to the symptoms in the acute cases of cachexia hypophyseopriva above recounted, a tendency to the deposition of fat was shown to occur in the more chronic states of secretory deprivation. All of these things, it will be seen, fall in with the view of a greatly lowered tissue oxidation or metabolism, and we came to recognize and to speak of the state as one akin to hibernation.[11]

Clinical Observations.—With the realization, through the experimental studies mentioned above, that the so called syndrome of Fröhlich is due to a deficient hypophysial secretion, it became apparent that other symptoms besides the adiposity and genital dystrophy of this clinical state were probably attributable to the same cause. Of these symptoms the drowsiness, which in extreme cases amounts to somnolence or lethargy, is the most important in our present connection. In many examples of the disease which we have observed, the condition has been so marked that the patients fall asleep during conversation or in the midst of an examination, and in a few instances somnolence has been so notably the most striking feature of the disorder that it has been the primary complaint.

Considerable attention was paid to this symptom in a volume[12] by one of us, dealing with the subject of pituitary disorders in general, and a number of clinical examples were given there, in one of which (Case XVI), a case of stationary hypophysial insufficiency, an extraordinary seasonal period of somnolence had recurred for several years.

The following case history will serve as a good illustration of the extreme degree of torpor which may accompany clinical hypopituitarism.

[10] The administration of glandular extracts to hypophysectomized animals or in clinical cases of hypophysial insufficiency tends to increase tissue oxidation, with a resultant rise in temperature. These thermic responses do not occur in controls.

[11] It may be added that studies by F. G. Benedict and J. Homans (The Metabolism of the Hypophysectomized Dog, *Jour. Med. Research*, 1911–12, xxv, 409) on the metabolism of an hypophysectomized dog have shown very much the same decrease in the respiratory quotient that has been observed in hibernating animals.

[12] Cushing, H., The Pituitary Body and Its Disorders, Philadelphia, 1912.

Interpeduncular Cystic Tumor of Pharyngeal Anlage. Characteristic Neighborhood Pressure Symptoms with Pronounced Glandular Manifestation of Hypophysial Insufficiency. Previous Diagnoses of Diabetes Insipidus, Dementia Paralytica, etc.—W. B., a business man, 48 years of age, entered the Johns Hopkins Hospital, Nov. 23, 1911, with the following symptoms notable in his complaint: constant headache, profound drowsiness, restricted vision, fainting spells, loss of potentio sexualis, great sensitiveness to cold, and polyuria.

His family record was remarkable merely from the standpoint of stature, several members exceeding 6 feet in height. His father was 6 feet tall and weighed over 200 pounds.

The story of his childhood and youth revealed nothing noteworthy, though he had not been particularly rugged and had been subject to headaches. He had married at the age of 30, and was the father of four healthy children.

Present Illness.—In 1905, six years before his admission, he received a severe blow in the face, which fractured his nose. He soon began to suffer from persistent headaches, and a noticeable loss of nervous energy subsequently became apparent. At about this time he became impotent.

In 1907 a diagnosis of diabetes insipidus was made, on the basis of a marked polyuria and polydipsia. In 1909 his vision began to fail. There was occasional diplopia, and a bitemporal hemianopsia was observed. At this time he had what was called a nervous breakdown.

With the further progress of his symptoms, a definite change occurred in his disposition. He neglected his business and financial affairs. He became forgetful, disoriented, and often more or less irresponsible. From the outset he was totally apathetic in regard to and seemingly oblivious of his illness. Osteopathy, a vigorous antiluetic régime, and other measures were tried without avail.

Drowsiness, first noticed a year previously, had become at the time of his admission the leading symptom. Not only did he pass most of the day in slumber, but on a number of occasions there was a wave of prolonged sleep lasting over several days, from which he could not be aroused. In two of these periods of deep somnolence, such a marked slowing of respiration occurred that recourse was had to artificial means for its continuance.

During his comparatively wakeful hours, he complained of extreme muscular enfeeblement and of chilliness even on the warmest days when others suffered from the heat. The skin was exceedingly dry; the hands and feet were always cold to the touch. For a long time the temperature had been persistently subnormal.

Physical Examination.—Briefly, this revealed a fair complexioned, well nourished man, 5 feet 9 inches in height, weighing 138 pounds, with thin hair and scant beard, and dry, parched skin. He was dull, apathetic, and drowsy, and unable to follow a consecutive train of thought. His speech was slow, drawling, and incoherent. The temperature (rectal) was 96.5° F., pulse 60, with a low blood pressure. The x-ray showed a sella of normal configuration. There was bitemporal hemianopsia with primary optic atrophy, which had progressed to near-blindness in the left eye. The Wassermann reaction was negative.

On Nov. 30, a week after his admission, he passed into a profoundly lethargic

state, with a systolic blood pressure below 100, a subnormal temperature of 96° F., slow pulse, and feeble, shallow respiration of Cheyne-Stokes type.

Under the misapprehension that the symptoms were manifestations of pressure, a right subtemporal decompression was performed. No anesthetic was used, and there was apparently complete insensitivity of the tissues. No increase in intracranial tension was disclosed.

His condition remained unmodified, and in desperation two days later, he was given a subcutaneous injection of pituitary (anterior lobe) extract. This was followed by the characteristic thermic reaction which we have described, with a rise of temperature to 101° F. Four hours after the injection he roused from his lethargy, seemed fairly rational, and asked for nourishment. This was the turning point in his critical condition, and for the succeeding two weeks he was kept in a fairly normal state of activity by repeated injections of extracts.

On Dec. 15 a transphenoidal operation with sellar decompression was performed, in the hope that the supposedly compressed gland might thus be relieved, and for some weeks there was a most unexpected betterment in his condition, both physical and mental, so that he was up, dressing, and caring for himself, and taking exercise independently. During the succeeding month he remained for the most part perfectly rational and physically active. Then followed an occasional period of somnolence, and finally he lapsed into his former state of lethargy.

The feeding of glandular extract in large doses proved unavailing, and recourse was again had to intramuscular injections. Under these injections, given daily, he invariably improved, but their frequent repetition caused so much muscular soreness that they had to be discontinued.

On Feb. 9, 1912, under primary anesthesia the pituitary gland of a still-born child was implanted in the subcortex at the seat of the decompression, and glandular administration was discontinued. There followed an unexpected and astonishing improvement in his condition. An analysis of his mental state at this time, made by Dr. Adolph Meyer, showed practically no deviation from the normal. His memory of past events was good and he had a peculiar subconscious recollection of his preceding months of somnolence.

He gained rapidly in strength, and on Mar. 10 he was discharged, apparently well, despite a tendency to nap in the afternoon, a continuance of polyuria, and unaltered neighborhood symptoms so far as the hemianopsia was concerned. He had been kept under observation during the full month in the expectation that the implanted gland might not actually have "taken."

On his departure he took a ten hour trip to his former home, attending personally to all the details of buying tickets, hotel registration, payment of bills, and so on. He met his many friends, remembered and kept appointments with promptitude, and went about independently,—indeed from a psychic standpoint seemed an absolutely normal individual and "like his old self."

More might be said concerning the return of somnolence, the patient's ultimate death, and the autopsy, which disclosed a characteristic infundibular cyst surmounting an almost completely destroyed hypophysis, but enough has been given to illustrate the hibernation-like symptoms which characterize some of these extreme states of hypopituitarism.

In all of our examples of pituitary disease associated with tumor or glandular enlargement we endeavor to distinguish clearly between the symptoms which are produced by pressure against neighboring structures and those due to a secretory disturbance on the part of the gland itself. Many of the symptoms which we are inclined to attribute to a primary hypophysial derangement, others have ascribed to an implication of hypothetical "nerve centers" at the base of the brain in the neighborhood of the gland. For example, in the case just cited it is true that the growth—a characteristic *Hypophysengangtumor* as described by Erdheim—compressed and distorted the structures in the interpeduncular space as well as the pituitary body itself; and were it not for the fact that quite comparable symptoms arise in cases of hypophysial disease in the absence of tumor, one might readily be led to accept the views of Erdheim, Aschner, Purves Stewart,[13] and others that some of the symptoms to which we have drawn attention are not evidences of dyspituitarism, but are due to pressure upon some predicated center in the tuber cinereum or elsewhere in the floor of the third ventricle. However, if the latter assumption were correct, the symptoms would hardly be improved by the administration of glandular extracts.

Without prolonging this report with further clinical illustrations, it is apparent that states of outspoken hypophysial insufficiency may be accompanied by symptoms comparable to those following the experimental deprivation of a large part of the gland in animals. The metabolic processes are at a low ebb, with a tendency in most individuals to the storage of fat, to an increased tolerance for carbohydrates, a subnormal temperature, slowed pulse and respiration, lowering of blood pressure, and often extreme somnolence.[14]

In addition to these symptoms, a notable expression of hypopituitarism is the more or less complete retardation of sexual development if the process antedates puberty, and the retrogressive changes

[13] Stewart, P., Four Cases of Tumour in the Region of the Hypophysis Cerebri, *Rev. Neurol. and Psychiat.*, 1909, vii, 225.

[14] One of us has commented elsewhere on the fact that the polyuria which often accompanies these states remains difficult of interpretation on the basis of glandular insufficiency (Cushing, H., Concerning Diabetes Insipidus and the Polyurias of Hypophysial Origin, *Boston Med. and Surg. Jour.*, 1913, clxviii, 901).

on the part of the reproductive organs and secondary sex characters if it affects adults. These disturbances are in all likelihood a consequence primarily of anterior lobe deficiency, as our experiments, to be reported under another title, would indicate.[15]

It can readily be seen that these experimental and clinical states possess many features in common with the condition of hibernation, —the deposition of fat, the somnolence, slow pulse and respiration, insensitivity, and subnormal temperature,—evidences, in brief, of a condition of lowered metabolic activity. We will return to the question of the activity of the reproductive glands.

Alterations in the Ductless Glands during Hibernation.

During the winter of 1912–13 we had the opportunity of studying the ductless glands of seven woodchucks[16] during and after the hibernating period. The carefully preserved glandular tissues of five of these animals (I, II, III, IV, and VII), which had hibernated in captivity, were forwarded to us through the kindness of Professor Sutherland Simpson, whose well known interest in the subject of hibernation has led to important studies,[17] but whose concern in this series lay in the direction of the nervous system. The two other animals (V and VI) were trapped in the open.

Professor Simpson's records state that his five woodchucks, all adults, fat and in good condition, were enclosed in their den without food, on December 9. One of the animals was sacrificed while dormant, on January 18, another on February 6, the third on February 22; the fourth was killed on March 15, soon after awaking, and the seventh on April 6, a month later. The fifth and sixth of the series were both caught for us in the open, on March 17 and 22, and killed

[15] We are coming to believe that not only is the factor of growth chiefly related to the pars anterior, but that this portion of the gland is more intimately associated with the activity or inactivity of the reproductive organs. We are only at the threshold of a satisfactory clinical differentiation between the constitutional effects of over- (perverted?) or undersecretion of the two lobes of the gland (Cushing, H., Concerning the Systematic Differentiation between the Two Lobes of the Pituitary Body, *Am. Jour. Med. Sc.*, 1913, cxlv, 313).

[16] The North American marmot (*Arctomys monax* or *Marmotta monax*).

[17] Simpson, S., The Food Factor in Hibernation (Preliminary Communication), *Proc. Soc. Exper. Biol. and Med.*, 1912, ix, 92; The Relation of External Temperature to Hibernation, *ibid.*, 1913, x, 180.

a few days later. Thus three of the animals were killed while hibernating, three soon after awaking, and the last a few weeks later.

The tissues received from Professor Simpson had all been preserved in the same fashion. The animals had been killed by cardiac transfixion and the organs immediately hardened *in situ* by aortic injections of saturated mercury bichloride in 10 per cent formalin. In the case of Woodchuck I the pituitary gland alone was received.

Woodchuck I.—Jan. 18, 1913. Animal found asleep. Rectal temperature 10.2° C. (50.3° F.). Hibernating for a period presumably of five or six weeks.

The block of tissue comprised a portion of the sphenoidal bone, the gland, and superimposed nervous tissues. The dural envelope and enclosed gland were carefully dissected away from the shallow sella turcica, mounted in paraffin, and serially sectioned in a sagittal direction.[18] The gland measured 3 mm. in its anteroposterior diameter.

Under the microscope the pars anterior shows a lack of the characteristic arrangement of the cells into cords and alveoli, though there are many well filled venous sinuses. The cells appear closely packed and possess deeply staining pycnotic nuclei, rich in chromatin and with but a small amount of surrounding, faintly pink staining, non-granular protoplasm. Particularly in the vicinity of the pars intermedia are the nuclei closely crowded together. Stained with hematoxylin and eosin the cells prove to be neutrophilic, none of them showing an especial affinity either for the acid or basic dye, such as characterizes the pars anterior cells under ordinary conditions.

There is a well formed cleft separating the pars anterior from the pars intermedia. The latter appears unmodified. The nuclei resemble those of the pars anterior, but they are surrounded by a larger amount of protoplasm with neutral staining affinities.

The pars nervosa also appears unmodified. The sections of the infundibular block show no apparent changes. The clusters of nerve cells of the tuber cinereum appear normal.

The finding of such definite histological changes in the pars anterior in this animal encouraged us to investigate the matter further, and when each of his remaining animals was killed, Professor Simpson was good enough to send us not only the pituitary body but fragments of the other ductless glands as well.

[18] In order to insure accuracy in the histological comparison between the glands of different individuals, it is essential not only that the sections be cut on the same plane, but that corresponding sections on this plane be compared. In this series of woodchucks, therefore, as in other animals and in man, median sagittal sections have been those of choice.

Owing to the cup-like shape of the enveloping anterior lobe these median sections show less of the pars anterior than do sections taken more laterally.

Woodchuck II.—This animal, a female, removed from the den and killed on Feb. 6, was still hibernating. Its rectal temperature was 14.8° C. The tissues were fixed as described, and the sphenoidal block, together with fragments of the other ductless glands, was sent to us.

Pituitary Body.—The gland was even smaller than that of Woodchuck I, measuring a scant 2.5 mm. in its anteroposterior diameter. In its histological appearance it corresponds precisely with that of the first animal, showing the same uniformly staining, closely packed pycnotic nuclei in the pars anterior (Figs. 1, 3, and 5).

Thyroid.—This shows no notable change. The acini are small, containing massive, deeply staining colloid, which fractures on section, making the tissue difficult to cut. The cells for the most part are flattened, though with cuboidal configuration around some of the acini. There is an abundance of intra-acinal fat, in which many clusters of hibernating gland cells appear.

Hibernating Gland.—The fragment of tissue marked "thymus" proves to be from the hibernating gland. The swollen cells hold fairly large, round nuclei and show an abundant granular protoplasm, free from vacuoles.

Adrenal.—The nuclei of both medulla and cortex appear somewhat shrunken, and more closely placed than normal, due to the relatively scant protoplasm. The nuclei of the medulla are somewhat irregular in shape.

Pancreas.—Here too the cells are moderately shrunken and the acini small. There are a few faintly staining secretion granules in the protoplasm (*Säure-violett* and *safranin O*). The nuclei are small and stain deeply. The islets are cellular, with scant protoplasm. There is an abundance of intra-acinal fat in the organ. The sections show a lymph gland with practically no eosinophils (compare No. VII).

Ovary.—There are a large number of primordial follicles and a few developing Graafian follicles, but none that are mature, and there are no corpora lutea. There is a small amount of interstitial tissue, but interstitial cells are not definitely recognized. The fimbriated extremity of the tube is lined by a low type of epithelium with very scant protoplasm.

The tissues of this animal (No. II) made it apparent, therefore, that hibernation is accompanied by histological changes in each member of the ductless gland series, though in none of them were the deviations from the normal as striking as in the pituitary body. The tissues of the third of the hibernating animals corresponded closely with the above, as follows.

Woodchuck III.—This animal, also a female, removed from the den while dormant and killed on Feb. 22, had a rectal temperature of 7° C. (44.6° F.). Presumably it had been hibernating for ten weeks, since about Dec. 9.

Pituitary Body.—The gland measured barely 3 mm. in its anteroposterior diameter. Histologically it corresponds in general with the glands of Nos. I and II. However, there is some tendency toward alveolar arrangement in the cells of the anterior lobe; they have somewhat more protoplasm and the nuclei are less pycnotic and are rounder and clearer than in Nos. I and II. A few of the cells show a slight affinity for acid stains.

Thyroid.—There is a large amount of intra-acinal fat. The acini themselves are similar to those described under No. II. The vesicles are possibly more distended with colloid, which stains uniformly pink. No cells of the hibernating gland type are observed.

Hibernating Gland.—This shows very little change from the hibernating gland of No. II, though the cells have begun to show some vacuolation.

Adrenal.—The gland is very small. The nuclei are shrunken, particularly those of the cortical cells, and compared with the awakening state (compare No. IV) the protoplasm is very scant.

Pancreas.—The acinus cells are distended and contain a large number of secretory granules. The nuclei are larger and more vesicular than in No. II, and occupy the base of the cells. The islets have a more open structure and are composed of loosely intertwined cell cords.

Ovary.—Much the same appearance as in No. II. A few follicles are somewhat more advanced, but none are mature, and there are no corpora lutea. The cells lining the fimbriated ends are taller and contain more protoplasm.

In the following example, the first of the awakened animals, a very obvious physiological reactivation was apparent, particularly in the anterior lobe of the hypophysis, but also in the cells of the adrenal. Unfortunately the sex of the animal was undetermined, as no block from the sexual glands was received.

Woodchuck IV.—This animal was found awake and active with a rectal temperature of 35° C., and was killed on Mar. 15. It had been incarcerated since Dec. 9 and had not had access to food during this period of ninety-six days. It had been observed on Mar. 8 and was then still asleep, so that its awakening must have been recent.

Pituitary Body.—The gland was macroscopically enlarged and swollen when compared with the glands of Nos. I, II, and III. It was thicker and measured 4 mm. in its anteroposterior diameter. Histologically the cells are swollen; their protoplasm is abundant, granular, and has begun to take differential eosinophilic and basophilic stains. A single mitotic figure was found.

The cleft contains colloid. The pars intermedia and pars nervosa are larger than in the earlier specimens; the cells are more active. The tissue of the posterior lobe is more distended and the nuclei are crowded farther apart, which makes the lobe itself appear less cellular.

Thyroid.—There is no particularly striking change, though the cells on the whole are more of the cuboidal type than in Nos. I and II. The intra-acinal tissue is composed of very scant connective tissue fibers, without fat tissue.

Hibernating Gland.—Sections were received from the three situations (cervical, thoracic, and subscapular), where the chief masses of this glandular structure occur.

The lobules appear smaller than those described under No. II, but there is no great change in the cells. The protoplasm is less abundant and stains less well, and many of the cells contain vacuoles of considerable size.

Adrenal.—Significant changes are apparent. The gland is larger than that of

No. II, and the cells of both medulla and cortex, but particularly those of the former appear swollen and show abundant protoplasm of granular structure. The nuclei are larger and more vesicular. The cortex shows more clearly than in No. III the normal characteristic division into zones.

Pancreas.—Very little change from No. III. The cells show a fair amount of protoplasm with abundant prozymogen granules at their base. No islets are seen on sections.

Ovaries or Testes.—Tissues not received.

Lymph Gland.—A single gland in one of the sections shows no eosinophilia.

The following animal (No. V) was trapped in the open in the neighborhood of Worcester, Mass., on March 17 and was forwarded to us for study. Its period of awakening may, therefore, have corresponded with that of the preceding animal (No. IV), though having been in natural surroundings it possibly became dormant earlier than December 9. It may also have had access to food during the winter, and the autopsy showed that it had fed on awaking and before capture, though subsequently it refused food. It may be noted that the winter of 1912–13 was not a very prolonged or severe one.

The animal was lively, fat, and appeared to be in good condition. It was sacrificed March 22, seven days later than No. IV.

Woodchuck V.—Mar. 22. An adult male, weighing 2,360 gm., very lively and fierce and apparently well nourished. Sacrificed by anesthetizing with ether and then bleeding from the carotid and femoral arteries. Immediate autopsy.

The abdominal cavity showed what appeared to be masses of adipose tissue in the mesentery and what was taken to be the omentum. This fat was white in appearance and of soft consistency. There was practically no subcutaneous fat. The cecum and large bowel contained food remnants, though the stomach and small intestine were empty.

Pituitary Body.—On opening the cranial cavity from above, the brain was elevated and the stalk of the hypophysis divided at the base of the third ventricle. The gland appeared to be considerably larger than those received from Professor Simpson, though the latter had not been seen in the fresh state. The posterior lobe was of a whitish appearance, very distinct from the dark pinkish pars anterior. Both lobes were flattened. The sella turcica was shallow. A block of tissue, including the sphenoidal bone and the floor of the sella, was cut out from the base of the skull and placed in Bensley fixative. The gland after fixation measured 4.5 mm. in its anteroposterior diameter, and 1.5 mm. in thickness.

Histological Appearance.—The cells of the pars anterior show the normal arrangement in cords; they are rich in protoplasm and have begun to show clearly their differential acid and basic staining reactions (Figs. 2, 4, and 6).

In the posterior lobe there is some increase in cellularity over the specimens previously studied.

Thyroid.—What was taken for the left thyroid, apparently with an accessory tag, was dissected out. It was not especially large and was of a faint pinkish yellow color. What appeared to be a parathyroid was evident at the upper pole and was left attached to the body of the gland. On account of bleeding from the right carotid, the tissues were suffused with blood and the right thyroid was not found. No thyroid tissue identified histologically.

Hibernating Gland and Thymus.—When the thorax was opened, in the position of the thymus there was found a very large, soft, brownish gland, of lobular structure, filling the upper mediastinum and embedded in a sort of adipose tissue. Nodules of similar tissue accompanied the intrathoracic vessels and extended downward along the course of the sympathetic trunk in the intercostal spaces as far as the diaphragm. Fragments of this tissue were preserved in various fixatives; the main bulk of the gland was preserved in formalin.

Histologically the gland differs greatly from the specimens previously studied. In many areas very little remains except the eccentric marginal nuclei of completely vacuolated cells. No thymic tissue identified in the sections.

Pancreas.—Diffuse lobules of the gland were found in a long process extending from the duodenum to the spleen, and small bunches of glandular tissue were seen in the mesentery extending downward almost to the kidneys. Pieces of the pancreas in the region of the splanchnics were taken out and placed in acetic-osmic-bichromate mixtures of 2 and 4 per cent osmic acid.

Histologically the gland is of normal appearance. The cells are large and filled with secretory granules.

Adrenals.—These appeared as small discrete glands of a greenish brown color. On section it was observed that a very large part of the cut surface was made up of pinkish white, soft, pulpy medulla. The cortex contained the pigment substance above described.

Histologically the medulla, when compared with the previous specimens, appears much enlarged in proportion to the cortex. Its cells are large, swollen, and compact. There is a much more distinct line of demarcation between the medulla and cortex, the lower zone of which appears flattened, as though the cells were compressed by the swollen medulla.

Testes.—The glands were large, and the structural elements were readily recognized. They measured about 1.75 cm. in length by 1 cm. in breadth. A surface scraping from the fresh section showed a few non-motile spermatozoa (?).

Histologically the gland shows an abundance of interstitial cells. The tubules contain a few spermatids, but no formed spermatozoa, nor are there any spermatozoa in the epididymis.

The next animal, a female, was likewise trapped in the neighborhood of Worcester, Mass., and was received a few days later than the preceding one.

Woodchuck VI.—An adult female, weighing 2,002 gm., active and seemingly well nourished. Rectal temperature 36.2°. Sacrificed on Mar. 27 by the same procedure as in No. V. The mammary glands were considerably hypertrophied and contained secretion.

Pituitary Body.—The gland appeared large, as in No. V. The two lobes were clearly distinguished. The sella was depressed, and its thin floor contained a small defect, as though produced by pressure atrophy.

Histologically a median section shows the pars anterior to be composed of large cells with differentially staining protoplasm, but with a preponderance of eosinophilic cells. The lobe is more vascular than those heretofore observed.

There is apparently some increase in the cellularity of the pars nervosa.

Thyroid.—What were taken for the thyroid lobes were removed, but the tissues proved to be symmetrically placed *lymph glands.* These show no excess of eosinophils (compare No. VII).

Hibernating Gland and Thymus.—Very much less in evidence than in No. VI. A small amount was apparent in the region of the thymus, with a few fine extensions downward on either side of the spinal column. Thymus not identified in gross.

Histologically the hibernating gland tissue shows greatly shrunken and vacuolated cells with small nuclei—a stage of absorption of the gland in advance of No. V. A small area of normal *thymus* appears on a few of the sections.

Pancreas.—Of normal appearance in gross.

Histologically the cells are larger than in the previous cases and are loaded with secretory granules. The islets present a normal appearance, with well developed cords and cells rich in protoplasm.

Adrenals.—In the fresh state, of a light green color, and somewhat larger than those of No. V.

On section the medulla appears less abundant in comparison with the cortex. When contrasted with the gland from a hibernating animal (*c. g.,* No. II), a very marked change is apparent, both in the size of the nuclei and amount of protoplasm. Next to the changes in the pituitary gland, the changes in the adrenal are the most striking.

Ovaries.—The right gland was considerably larger than the left. On section it was of a more pinkish color and contained what appeared to be numerous corpora lutea. The left ovary was whiter in appearance and showed one nodular elevation, apparently a corpus luteum.

Histologically the right gland is largely made up of several corpora lutea, with but little interstitial substance containing a few immature follicles.

Fallopian Tubes.—The uterus appeared to be thickened, edematous, and vascular. At uniform intervals there were small dilatations suggesting early pregnancy.

Histologically the tubes show a thick, vascular endometrium with high columnar epithelium.

Whether or not this animal was pregnant was not determined. The condition of the tubes and ovaries resembled closely that which will be described elsewhere as a consequence of pituitary feeding.

The following animal, the last of Professor Simpson's series, was found awake on March 15, when No. IV was sacrificed; but on March 22, when again observed, it was in a condition of semihiber-

nation, with a rectal temperature of 15° C. It was observed again on March 29 and was found to be in the same dormant condition, with a rectal temperature of 12.7° C., and when replaced in the den, a dish of water and some cabbage were set beside it. On April 5 it was found to be awake and active, with a temperature of 38° C. (100.6° F.) and a body weight of 1,150 grams. A runway was attached to the den, so that the animal was free to move about, and it was abundantly supplied with food and water.

When observed on April 16 the rectal temperature was 35.8° C. (96.5° F.), body weight 1,500 grams. On May 5 rectal temperature 37.7° C. (100° F.), body weight 1,650 grams.

Woodchuck VII.—Female; killed May 5. Tissues from Professor Simpson were fixed as in the early series. It may be noted that it had been actively awake for a month and had put on weight. In comparison with our other animals it was small and may possibly have been younger.

Pituitary Body.—The gland, though somewhat smaller than the foregoing, shows characteristic anterior lobe differential staining reactions. The pars intermedia and pars nervosa are unchanged.

Thyroid.—The vesicles are surrounded by possibly a somewhat more cuboidal type of cell on the average than were those in No. II, though there appears to be very little difference from the winter state.

Hibernating Gland.—Here the changes are very marked from the winter state. The cells have lost their granular appearance and sharp outlines and are more ragged in form. The nuclei, from loss of protoplasm, appear more closely packed and smaller, with more dense chromatin. The cells are considerably vacuolated.

Pancreas.—No changes were observed. Special stains for zymogen granules, etc., were not employed.

Adrenals.—The cortex appears much thickened in comparison with the medulla. The cells of the medulla are large and active in appearance. A slight differentiation is evident between the zones of the cortex.

Ovary.—Gland apparently active, with increase of interstitial cells. Fully ripened, large Graafian follicles are present, and there is one large corpus luteum.

Lymph Gland.—A gland present in the tissues from the pancreas, and another present in the tissues from the hibernating gland in the neck, show an extraordinary preponderance of eosinophils.

Comment.—Though the observations on this series of seven animals are somewhat fragmentary,[19] and though more or less definite

[19] We had hoped to supplement these observations by the study of a series of animals during the winter of 1913–1914. We were able to secure for the purpose, however, only one woodchuck; but at the same time three hedgehogs and three raccoons were also interned. The winter was a particularly severe one and all four animals succumbed to the low temperature. Our provisions for

evidences of a pluriglandular insufficiency are apparent when the waking is contrasted with the dormant state, nevertheless the most striking histological changes occur in the anterior lobe of the pituitary gland. These changes, in brief, consist of a loss of the characteristic cellular topography of the pars anterior, and, in the case of the individual cells, of a shrinkage of both the nuclear and protoplasmic substance of the cells, with complete loss of the characteristic histological picture of the active gland,—namely, the differential staining qualities of the granular content to acid and basic dyes, —qualities which reappear at the end of the dormant period. It may be added that in only one of our specimens have we observed the karyokinetic figures which Gemelli (see below) has described as an especial feature of the awakening state. This may be for the reason that we have not happened upon the exact time when, after awakening, the cell division is particularly active. Significant of Gemelli's findings, however, is the fact that the writers, except in Case IV of this series, have never observed mitosis in any of the countless pituitary glands of the lower animals they have examined.

In view of the close interrelation of the pituitary body and the sexual glands it is unfortunate that we were deprived of the latter organs in Nos. I and IV, and that four of the remaining five animals were females. It is our presumption that very marked activation occurs in the reproductive organs when the animal emerges from the dormant state, and it is tempting to attribute this to the influence of the functionally reactivated pars anterior. This conjecture is supported by the experiences of one of us (Goetsch) with the

proper hibernation were doubtless inadequate. Food was supplied so that the animals might eat as they desired, and they all did so with more or less regularity. The woodchuck, which was a male, survived a particularly cold spell during which the other animals perished, but at no period had it definitely hibernated. On Jan. 12 it was fairly lively, with a rectal temperature of 32.5° C., weight 1,807 kilos. It was sacrificed. There was very little fat, but a large amount of hibernating gland. The pituitary body resembled the glands of Woodchuck III of the series. It was small and the cells were somewhat closely packed, and some of them took differential stains. The testes showed active cell division of spermatogenous cells, but there were no spermatozoa; the interstitial cells were abundant. There were no notable changes in the other glands. No changes of recognizable significance were made out from the tissues of the raccoons or hedgehogs, and no tissue resembling hibernating gland tissue was positively identified in any of them.

feeding of pars anterior extract and its effect in stimulating reproductive activity,[20] which will be the subject of a subsequent paper.

The Observations of Gemelli and Salmon.

As far as we are aware, only two writers, both of them Italians, have approached this subject in such wise that their views and observations are at all pertinent to this discussion.

In 1905 a monograph on sleep was published by Salmon[21] in which the view was advanced that physiological sleep is due to an internal secretion of the pituitary gland. In later papers, published in French,[22] this view was still further elaborated.

In 1906 an important paper appeared in Italian, written by Agostino Gemelli,[23] in which the histological changes occurring in the pituitary gland of the hibernating marmot were fully described for the first, and as far as we can learn, for the only time. In a second paper[24] he refutes the contentions of Salmon and ascribes to the hypophysis in cooperation with the other ductless glands the function of the neutralization of toxins.

It is our impression that the observations of these writers are in entire accord with our own, but that their conclusions, particularly those of Salmon, in so far as they were drawn from clinical observations, are based on wrong premises.

Salmon advanced the opinion that the hypophysis is actually a center for sleep, normal physiological sleep being explained on the basis of an internal secretion from the gland which has some vasomotor or autotoxic effect on the nervous system: in other words, that physiological sleep is essentially dependent upon the secretion of the pituitary gland.

As an argument favoring this view he gives a list of disorders in which drowsiness is commonly observed, and in many of which there occurs some hypophysial alteration. Strangely enough, however, he attributes drowsiness to conditions of glandular hyperplasia, accompanied by hyperfunction, whereas insomnia is ascribed to states of glandular atrophy with functional insufficiency. This, it will be seen, is precisely the reverse of the view which is now commonly

[20] Certain clinical experiences are also in accord with this view: for the feeding of hypophysial extracts over prolonged periods to patients who are the victims of hypopituitarism not infrequently arouses sexual activity after periods of amenorrhea or impotence.

[21] Salmon, A., Sull' origine del sonno. Studio delle relazioni tra il sonno e la funzione della glandula pituitaria, Florence, 1905.

[22] Salmon, A., Le sommeil pathologique; l'hypersomnie, *Rev. de méd.*, 1910, xxx, 765; La fonction du sommeil. Physiologie, psychologie, pathologie, Paris, 1910.

[23] Gemelli, A., Su l'ipofisi delle marmotte durante il letargo e nella stagione estiva, *Arch. p. le sc. med.*, 1906, xxx, 341.

[24] Gemelli, A., Nuove osservazioni su l'ipofisi delle marmotte durante il letargo e nella stagione estiva; contributo alla fisiologia dell' ipofisi, *Biologica*, 1906, i, 130.

accepted. At the time of Salmon's publication, it may be recalled, there was no satisfactory differentiation of the clinical expressions of hyper- and hypopituitarism.

Gemelli was quick to see the falsity of Salmon's position, for he says: "If Salmon's hypothesis were correct we should expect to find an increase in the activity of the pituitary gland during hibernation, for only in this way could we confirm the view that physiological sleep is due essentially to the secretion of the glandular portion of the pituitary body. But we have really found the contrary to be the case—*viz.*, a lowering of the proper activity of the organ characterized by a decrease of the secretory cells. After the marmots awake we find a regeneration of the glandular tissue, with a great number of karyokinetic figures and an increase in the number of eosinophil cells. These observations point to an organic renovation of the gland and an increase in its activity, and therefore refute entirely Salmon's hypothesis."

If Salmon had reached the opposite conclusion, which assuredly he would have done had he been in possession of the facts before us today, namely, that drowsiness or somnolence is induced by a deficiency rather than an increase in hypophysial secretion, it is possible that Gemelli might have found reason to ascribe the somnolence of his marmots to the changes in the gland which he was the first to observe. As it was, Gemelli interpreted his findings as an argument in support of the view, which has become generally accepted, that the gland is of prime physiological importance and is not a rudimentary structure. With many other French and Italian investigators he contends that the ductless glands—adrenal cortex, thyroid, parathyroid, and hypophysis—are organs whose chief function is the neutralization of toxins, not only such as are furnished by organic, inorganic, and bacterial poisons, but those the products of metabolism as well. To quote from him: "During hibernation metabolism is decreased and therefore we have less toxic substances. When the animals awake in the spring the vegetative functions are resumed, and we therefore find a very great increase of toxic substances, corresponding to which the changes described in the pituitary gland occur." Gemelli's conclusions are as follows:

1. The hypophysis of the marmot follows the general law which governs the other organs during hibernation and the spring awakening.

2. The decrease in cyanophil cells during hibernation, the appearance of numerous karyokineses, and increase in cyanophil cells during the spring awakening, serve to confirm the hypothesis that the function of the glandular lobe of the hypophysis cooperates with the other ductless glands in neutralizing toxins.

3. The anterior part of the glandular lobe of the hypophysis cannot be regarded as a hypothetical center of physiological sleep.

Hibernation in General.

In physiological adaptation to periodic conditions unfavorable to life, of which a seasonal diminution of food supply is perhaps the most important element, certain animals have acquired a capacity for seasonal lethargy which appears to be but a form of prolonged diurnation, just as diurnation is an exaggeration of normal sleep. A low external temperature would appear to be merely a predisposing factor in hibernation, just as it is in the case of normal

sleep, but from what we now know of æstivation the external temperature in itself can hardly be regarded as essential to the process.

Of the fur-bearing animals of the temperate latitudes the marmot, as far as is known, shows the phenomenon of hibernation in its most outspoken form. Winter sleep, however, is a peculiarity of other so-called poikilothermic animals, such as the dormouse, hedgehog, bat, and prairie-dog; and it is alleged that raccoons, skunks, badgers, bears, and even some squirrels and other animals, such as chipmunks and gophers, which store their winter provender, at least have the capacity of passing the months of scant food supply in a semihibernating condition. It is a physiological characteristic which has never been acquired by birds, due in all likelihood to their ability to migrate to more favorable surroundings.

Many of the animals which undergo a period of hibernation or semihibernation in their normal habitats when transplanted to other surroundings and furnished with food may not lapse into their wonted months of continuous sleep, but may remain more or less active; and this is true even of the typically hibernating marmot. It is conceivable that under these changed conditions they might grow out of the habit, though unquestionably they would still retain the capacity to hibernate were the original conditions resumed. It may be added, however, that certain animals when transplanted retain their habit of periodic somnolence. This is said to be true of the tenec of Madagascar, an animal belonging to the same order as the hedgehog. The tenec undergoes what is rare in mammals, namely, a period of summer sleep, and the specimens in captivity in the London Zoölogical Garden are said to have continued in their regular seasonal sleep even in the northern latitude.

The same physiological changes, though of brief duration, appear to take place in animals which have the habit of diurnation, a condition which apparently differs from hibernation only in its brevity. This is true of the bat, for example. Dormice, too, awake every twenty-four hours merely to feed; and the sleep of hedgehogs may last for two or three days at a time. It is alleged that during diurnation in these animals, respiration practically ceases and the temperature falls to about that of the surrounding medium and rises again with their awakening. Indeed, the fall in temperature which occurs during the natural sleep of all non-hibernating species[25] suggests that no sharp line of demarcation separates the two conditions.

Similar seasonal physiological rhythms may possibly affect other mammals besides those mentioned,[26] and attention was drawn, in the course of our canine hypophysial studies during three or four consecutive winters, to the fact that

[25] Simpson, S., and Galbraith, J. J., An Investigation into the Diurnal Variation of the Body Temperature of Nocturnal and Other Birds, and a Few Mammals, *Jour. Physiol.*, 1905–06, xxxiii, 225.

[26] Among cold-blooded animals, both vertebrate and invertebrate—snakes, lizards, tortoises, frogs, toads, snails, and so on—the phenomenon is of quite general occurrence. Hibernation in the carnivora is limited to the *Arctoidea*. The single canine example is apparently the raccoon-dog of Japan, which is said (Radde) to hibernate if food has been sufficiently plentiful to enable the animal to store sufficient fat.

in the midwinter months hemodynamic and other reactions to the injection of hypophysial extracts often did not occur or were far less pronounced than at other times of the year. It was suggested in explanation, (1) that a seasonal change in the animals experimented upon caused the administration of the extracts to fail to give the usual responses, or (2) that the glands of the animals from which the extracts had been prepared were in a condition of relative seasonal inactivity and that the extracts consequently were less active in their effect. It is possible that we may thus account for some of the discrepancies in the results obtained by various observers of the physiological reactions of prepared extracts.[27]

Even man appears to have a certain capacity for what may be called winter sleep, as has been shown by the experiences of those in the arctic regions; and it is possible that such a capacity might be further developed, as seems to be the case with the peasants in certain parts of Russia, who during the winter months, when there is a scarcity of food, pass weeks at a time in a somnolent state, arousing once a day for a scant meal.

Unquestionably the underlying factors in hibernation are extracorporeal ones relating to food supply, but it may be presumed that animals subjected to seasonal periods of food deprivation have acquired the capacity to survive, partly through the preliminary storage of tissue fuel and partly through the ability to conserve that fuel so that its combustion is extremely slow. Nothing would be so likely to favor these conditions as a physiological period of inactivity of those glands which are concerned with tissue metabolism, of which the pituitary body has been shown to be a striking example.

That deprivation of food and exposure to cold cannot be the only factors is shown by the fact that such an animal as the woodchuck in its natural surroundings often goes into winter quarters long before the onset of cold weather and emerges again at the vernal equinox, even though existing climatic conditions may be most unfavorable for the securing of food. Moreover, it has been found impossible to force hibernation by the withdrawal of food and exposure to cold out of season.

The occurrence of such a seasonal wave of inactivity on the part of certain

[27] R. Hunt (The Effects of a Restricted Diet and of Various Diets upon the Resistance of Animals to Certain Poisons, *Bull. Hyg. Lab., U. S. P. H.*, 1910, No. 69) called attention to the great variation in the resistance of mice and guinea pigs to certain poisons and attributed them to seasonal variations in the activity of the thyroid glands. A. Seidell and F. Fenger (Seasonal Variation in the Composition of the Thyroid Gland, *Bull. Hyg. Lab., U. S. P. H.*, 1914, No. 96) showed that there are, as a matter of fact, seasonal changes in the weight as well as in the iodin content of the thyroid glands of beef and sheep. G. B. Roth (Pituitary Standardization. A Comparison of the Physiological Activity of Some Commercial Pituitary Preparations, *Bull. Hyg. Lab., U. S. P. H.*, 1914, No. 100) has called attention to the wide variability and the physiological activity of commercial pituitary extract, but has assigned no reasons for the differences.

Dr. Hunt expresses the belief that pituitary preparations vary on account of the difference in the methods of manufacture, rather than on account of the difference in the original gland.

members of the ductless gland series as our theory of hibernation predicates is no more remarkable than other periodic waves of inactivity or activity in the case of other glands. This is particularly true of the glands directly concerned with reproduction,—glands which have been shown to have a very close functional interrelationship with the pituitary body.

SUMMARY.

A train of symptoms, coupled with retardation of tissue metabolism and with inactivity of the reproductive glands, not only accompanies states of experimentally induced hypophysial deficiency, but is equally characteristic of clinical states of hypopituitarism. The more notable of these symptoms are a tendency, in the chronic cases, toward an unusual deposition of fat, a lowering of body temperature, slowing of pulse and respiration, fall in blood pressure, and oftentimes a pronounced somnolence.

These symptoms bear a marked resemblance to the physiological phenomena accompanying the state of hibernation which have heretofore been unsatisfactorily ascribed solely to extracorporeal factors; namely, a seasonal deprivation of food and low temperature.

In a series of hibernating animals (woodchucks) it has been found that during the dormant period histological changes are apparent in many of the ductless glands. The most notable of these changes occur in the pituitary body, as previously observed by Gemelli. The gland not only diminishes in size, but the cells of the pars anterior in some animals at least completely lose their characteristic staining reactions to acid and basic dyes. At the end of the dormant period the gland swells, and as the cells enlarge they again acquire their differential affinity for acid, basic, and neutral stains, and at the same time karyokinetic figures may appear.

CONCLUSIONS.

On the basis of these observations hibernation may be ascribed to a seasonal physiological wave of pluriglandular inactivity. The essential rôle may perhaps be ascribed to the pituitary body, not only for the reason that the most striking histological changes appear in this structure, but also because deprivation of the secretion of this gland alone of the entire ductless gland series produces a group of symptoms comparable to those of hibernation.

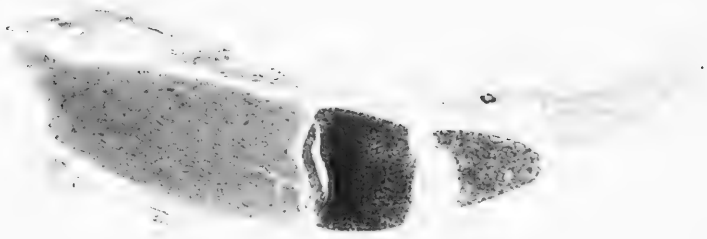

FIG. 1.

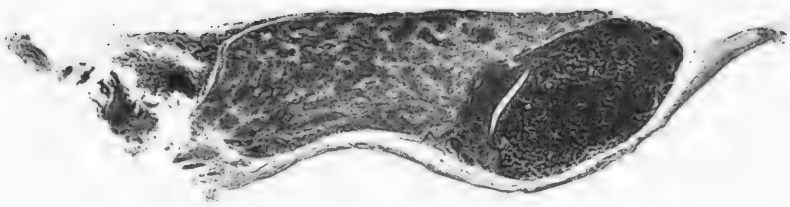

FIG. 2.

(Cushing and Goetsch: Hibernation and the Pituitary Body.)

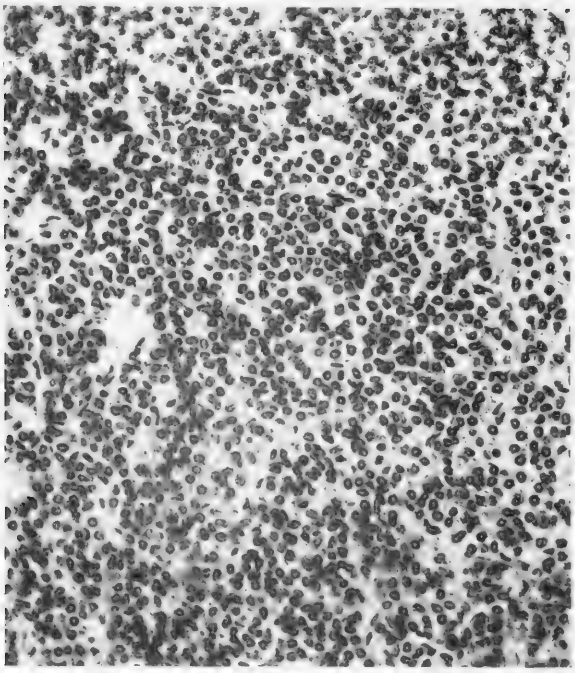

FIG. 3.

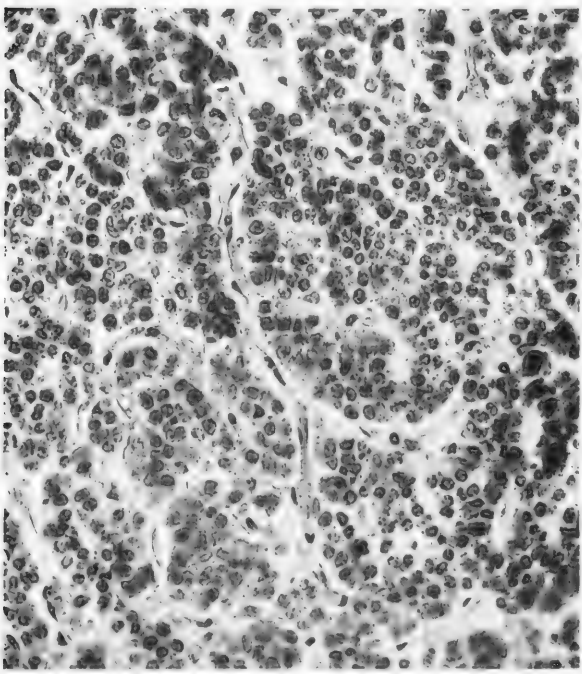

FIG. 4.

(Cushing and Goetsch: Hibernation and the Pituitary Body.)

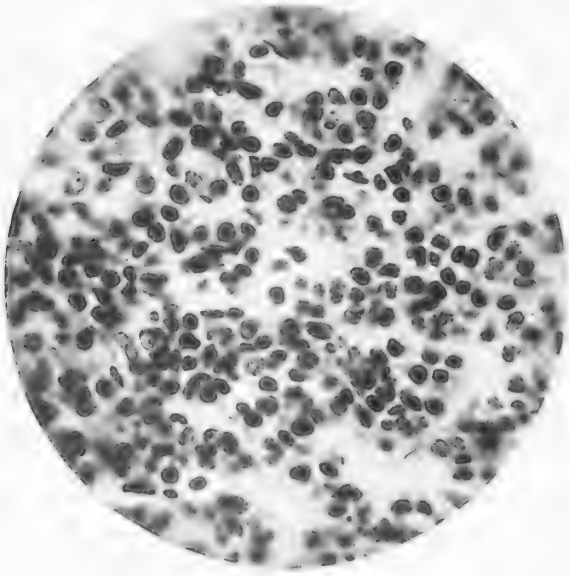

FIG. 5.

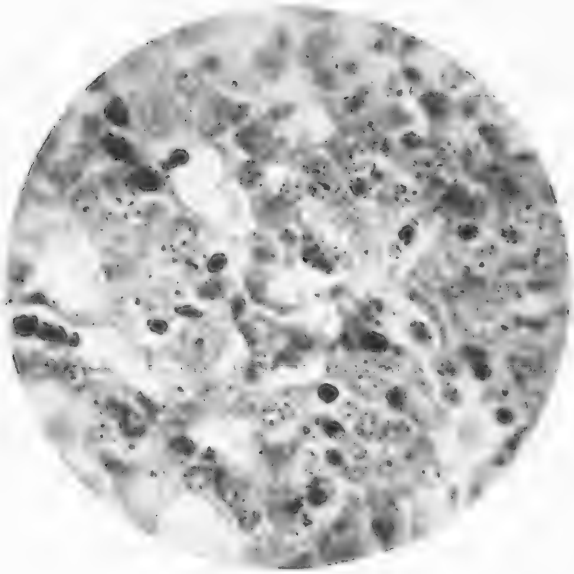

FIG. 6.

(Cushing and Goetsch: Hibernation and the Pituitary Body.)

EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Median section of the pituitary gland of an hibernating woodchuck, killed Feb. 15, 1913 (No. II). Pars anterior broken.

Fig. 2. Median section of the pituitary gland of a woodchuck (No. V), killed Mar. 22, 1913, a few days after awakening.

Plate 2.

Fig. 3. Undifferentiated anterior lobe cells of the hibernating state (Woodchuck II), Feb. 15, 1913. Hematoxylin and eosin. × 300.

Fig. 4. Well differentiated anterior lobe cells of an animal on awakening (Woodchuck V), Mar. 22, 1913. Hematoxylin and eosin. × 300.

Plate 3.

Fig. 5. Higher magnification of the cells of the hibernating state (Woodchuck II, Feb. 15, 1913). *Säureviolett* and *safranin O*.

Fig. 6. Higher magnification of the anterior lobe cells of the awakening state (Woodchuck V, Mar. 22, 1913). *Säureviolett* and *safranin O*.

LATE POISONING WITH CHLOROFORM AND OTHER ALKYL HALIDES IN RELATIONSHIP TO THE HALOGEN ACIDS FORMED BY THEIR CHEMICAL DISSOCIATION.

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PLATES 4 AND 5.

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It is well known that the prolonged administration of chloroform may be followed by certain well marked morphological changes in the tissues, most conspicuous of which are edema, fat infiltration, multiple hemorrhages, and necrosis of the central portion of the liver lobule. These changes have been extensively studied; and excellent bibliographies may be found in articles by Bevan and Favill,[1] Wells,[2] Whipple and Sperry,[3] Howland and Richards,[4] and others. In this article, therefore, no detailed discussion of the changes will be given. However, in spite of the large amount of study that has been devoted to the lesions, there has apparently been little effort to analyze the factors involved in their production. At the present time there is no adequate explanation of how these changes are produced. A satisfactory explanation would be important not merely

[1] Bevan, A. D., and Favill, H. B., Acid Intoxication, and Late Poisonous Effects of Anesthetics. Hepatic Toxemia. Acute Fatty Degeneration of the Liver Following Chloroform and Ether Anesthesia, *Jour. Am. Med. Assn.*, 1905, xlv, 691.

[2] Wells, H. G., Chloroform Necrosis of the Liver, *Arch. Int. Med.*, 1908, i, 589.

[3] Whipple, G. H., and Sperry, J. A., Chloroform Poisoning. Liver Necrosis and Repair, *Bull. Johns Hopkins Hosp.*, 1909, xx, 278.

[4] Howland, J., and Richards, A. N., An Experimental Study of the Metabolism and Pathology of Delayed Chloroform Poisoning, *Jour. Exper. Med.*, 1909, xi, 344.

in connection with the action of chloroform itself but rather because of the light which it would throw on the nature of the fundamental processes involved, which without doubt are identical with those concerned in poisonings of the body with a large group of toxic substances, including other narcotics, arsenic, salvarsan, phosphorus, and probably most bacterial poisons. Evidence will be brought to show that in chloroform poisoning the liver necrosis is produced chiefly by the action of acid (largely probably by hydrochloric acid which is formed in the metabolic destruction of chloroform), and this ability to produce liver necrosis is a general property of alkyl halides, all of which probably yield halogen acids in their breakdown in the body. Reasons will also be brought in support of the view that the accompanying cloudy swelling, fat infiltration, hemorrhages, and edemas are also acid effects. These changes, however, are not limited to intoxications with substances which can split off mineral acids, but they may follow the administration of any substance that can cause tissue asphyxia with its attendant accumulation of organic acids.

The ease with which extensive liver necrosis is induced by a two or three hour narcosis with chloroform, and the failure to obtain it by a narcosis with ether of three or four times that duration, suggest strongly that this difference in the behavior of the two substances depends upon either a difference in molecular action or a difference in products formed during their breakdown.

When chemically pure chloroform is exposed to the action of sunlight at room temperature it is oxidized to phosgene and hydrochloric acid. The end reaction may be expressed thus:

$$CHCl_3 + O \rightleftharpoons COCl_2 + HCl.$$

This oxidation occurs so easily that it has become necessary for manufacturers of chloroform for anesthetic purposes to add alcohol to it to prevent its decomposition.[5] Phosgene in the presence of

[5] Baskerville has shown that the alcohol protects the chloroform because it is more easily oxidized than chloroform, and hence when, as in a stoppered bottle, there is only a limited amount of oxygen present, the alcohol uses all the available oxygen for its own oxidation (Baskerville, C., and Hamor, W. A., The Chemistry of Anesthetics. IV. Chloroform, *Jour. Indust. and Eng. Chem.*, 1912, iv, 362).

water is decomposed into carbon dioxide and two molecules of hydrochloric acid. This hydrolysis may be expressed thus:

$$COCl_2 + HOH \rightleftarrows CO_2 + 2HCl.$$

It is at once apparent, therefore, that if one molecule of chloroform is oxidized in the presence of water, three molecules of hydrochloric acid may be formed. The liberation of such a strong inorganic acid in the tissues could very easily produce necrosis and other acid effects; and since three molecules of the acid are formed from each one of the chloroform, it might easily be supposed that the oxidation of only a small amount of chloroform would suffice to produce a considerable effect on the tissues. Moreover, the well known facts that the liver is the organ which most strikingly manifests the chloroform necrosis, and that this organ is also the site of a most active metabolism, harmonize well with this hydrochloric acid theory.

With these facts and considerations as the basis for departure, the hypothesis was subjected to a series of experimental tests which may be outlined as follows.

1. A study was undertaken of the morphological changes induced by hydrochloric acid, with special reference to the liver. 2. Attempts were made to demonstrate free hydrochloric acid in the necrotic areas in the livers of animals poisoned with chloroform. 3. Observations were made on the relative necrosis-producing power of different chlorine substitution products of methane (*e. g.*, dichlormethane, chloroform, and tetrachlormethane), which on theoretical grounds could be considered to yield different amounts of hydrochloric acid in their breakdown. 4. The inhibiting effect of alkali was studied. 5. Attempts were made to produce the typical picture of chloroform poisoning by other alkyl halides of the same type as chloroform, *viz.*, bromoform ($CHBr_3$) and iodoform (CHI_3), which might be expected to give analogous products in their breakdown, with, however, the liberation of hydrobromic acid and hydriodic acid, respectively, instead of hydrochloric acid. 6. Experiments were made to ascertain whether or not morphological effects like those produced by chloroform can also be induced by alkyl halides in general, and whether these substances are decomposed in the body in such a way that the corresponding halogen acid is liberated.

7. A comparative study of the lesions produced by chloral hydrate and chloroform was made, since the former substance yields practically no hydrochloric acid in its metabolic breakdown, although it contains the same number of chlorine atoms as chloroform.

EXPERIMENTAL.

The Tissue Changes Induced by Administration of Hydrochloric Acid.

Oral and intraportal administrations of hydrochloric acid in suitable concentrations were followed by edema, hemorrhages, necrosis, and increased fat accumulation in the liver. When injected intraportally in dogs in relatively high concentrations (10 to 25 cubic centimeters of 0.5 (N/7) to 1 per cent (N/3.5) solutions), hydrochloric acid produced extensive liver necrosis involving large areas of many lobules about equally. When given in lower concentrations (*e. g.*, 0.37 per cent (N/10)), it produced edema and other degenerative changes as well as numerous subcapsular hemorrhages. The parenchymatous changes were most conspicuous at the periphery of each lobule. This was to be expected since, because of the arrangement of the blood supply, the periphery would be the first part of the lobule reached by the acid. The degenerative changes consisted of pycnosis and fragmentation of the nuclei with swelling of the cells. It was felt that these were precursors of necrosis. When several administrations were given by mouth to rabbits in concentrations of 1 (N/3.5) or 2 per cent (N/1.8) at intervals of from twelve to twenty-four hours, the animals usually died after the third or fourth administration. They showed extensive hemorrhage in the mucosa of the stomach and duodenum, large fatty livers, and swollen kidneys.

Microscopically, by the use of Sudan III, the fat in the liver was found chiefly around the central veins. In short, all of the marked morphological changes induced by chloroform have been seen to follow the administration of hydrochloric acid alone. They were, however, differently distributed. The liver necrosis appeared at the peripheries of the lobules instead of at the centers, as in chloroform poisoning, but this difference in location is not regarded as important, since a discussion of the site of the necrosis in chloroform poisoning involves the question of the site of the greater formation of hydrochloric acid together with that of the relative susceptibility of the central and peripheral parts of the lobule to its effects. These points will be further elaborated in the discussion.

The production of fatty changes in the liver by the use of hydrochloric acid is in harmony with a statement of Leathes,[6] that as an effect of "the action of mineral acids an active mobilization of the fat reserves may occur . . . and the liver cells are found loaded with fat of a low iodine value." Whether or not this mobilization of fat is to be considered as due to a primary effect of the acid or to the cellular asphyxia induced by the acid will not be discussed in this paper.

The extensive edema which follows the administration of hydrochloric acid confirms the well known observations of Fischer on the imbibition of water by tissue colloids under the influence of acids generally. The production of hemorrhages harmonizes with the frequency of their occurrence after tissue asphyxia, with its attendant formation of acid. Doyon[7] and his pupils first (1905–1906) called attention to the probability that fibrinogen is formed mainly in the liver and that interference with normal liver function induces a diminished coagulability of the blood, a conclusion which later was confirmed by Whipple and Hurwitz.[8] In a previous paper in which it was shown that the various hemorrhagic diseases of the newly born are probably expressions of an asphyxial process,[9] we stated that this hemorrhagic tendency might be due to a "more fundamental and wide-spread change, as a result of which not only fibrinogen, but innumerable other proteins tend to remain in solution or to pass into solution, with the result that apart from diminished blood coagulability there is a great reduction in the firmness of the vessel walls." Typical protocols follow.

Experiment 1.—Normal dog, weighing 5 kilos. 2.00 p. m. Under ether anesthesia abdomen opened and 2 cc. N/10 (0.37 per cent) hydrochloric acid injected into radicle of portal vein (branch of superior mesenteric). Marked dyspnea and muscular spasms occurred two minutes later. 2.30 p. m. 2 cc. again injected, followed by same symptoms. 2.45 p. m. Another injection of 2 cc. Few small hemorrhages noted on surface of liver. 3.00 p. m. Another injection of 2 cc. Hemorrhages more numerous; also appearing on wall of stomach. When liver is stroked with handle of scalpel a line is marked which immediately becomes dark red (as if hemorrhagic). 3.30 p. m. After another injection of 2 cc., marked respiratory spasm occurred with muscles of chest rigid. Artificial respiration necessary. Hemorrhages increasing. Stomach greatly distended. 4.00 p. m. Another injection of 2 cc. 5.00 p. m. Dog died. Stomach enormously distended with gas. Wall very hemorrhagic. About 10 cc. of unclotted blood in abdomen. On cutting the small vessels there is little tendency of the blood to coagulate. Several subcapsular hemorrhages on the surface of the liver were noted, varying from pin-head to 4 or 5 mm. in size. No gross increase in liver fat.

Experiment 2.—Dec. 23, 11.30 a. m. Two adult rabbits each given 50 cc. of

[6] Leathes, J. B., The Fats, London, 1910, 111.

[7] Doyon, M., Modifications de la coagulabilité du sang consécutives à la destruction du foie. Pathogénie des hémorragies symptomatiques des affections du foie, *Jour. de physiol. et de path. gén.*, 1905, vii, 639.

[8] Whipple, G. H., and Hurwitz, S. H., Fibrinogen of the Blood as Influenced by the Liver Necrosis of Chloroform Poisoning, *Jour. Exper. Med.*, 1911, xiii, 136.

[9] Graham, E. A., The Pathogenesis of the Hemorrhagic Diseases of the New-Born, *Jour. Exper. Med.*, 1912, xv, 326.

2 per cent hydrochloric acid by stomach tube. Marked dyspnea followed. The same amounts of acid were again given on Dec. 26, Jan. 4, Jan. 10, and Jan. 15.

Jan. 16. One rabbit was found dead in the morning.

Autopsy.—The liver was large and yellowish, with very distinct lobular markings. Frozen sections stained with Sudan III showed very extensive fat accumulation, most marked around the central veins. Kidneys were swollen and pale brown. Microscopically they showed marked parenchymatous degenerative changes, especially marked in the convoluted tubules. A few casts were evident.

Jan. 16. 1.30 p. m. The other rabbit was given the same amount of acid again. Dead at 4.30 p. m.

Autopsy.—The liver was moderately fatty. The stomach and duodenum were markedly hemorrhagic; the duodenum was empty and firmly constricted throughout its whole length. The kidneys were swollen and pale.

Attempts to Recognize Hydrochloric Acid in the Necrotic Areas in the Liver.

The practical identification of free hydrochloric acid depends upon the recognition of (1) free hydrogen ions and (2) free chlorine ions. It is obvious then that efforts aiming at the direct proof of the presence of hydrochloric acid in the tissues are necessarily complicated by the fact that both ions are always present. Therefore, at best, only circumstantial evidence can be brought to show the presence of free hydrochloric acid. However, the following observations were made.

It is possible to show a high hydrogen ion content in the central, necrotic portions of the liver lobules if fresh unstained sections are treated with indicators which do not change when placed in contact with equally fresh normal tissues in the same way. In this work most of the observations were made with neutral red and phenylated Nile blue. In every case the reaction between the indicator and tissue was much more marked in the central (necrotic) portion than at the periphery of the lobule. In some instances the tint assumed by the neutral red was a deep rose, approximating that which is obtained with a concentration of acid represented by $H^+ = 10^{-6}$. Often the central portion was distinctly red, while the periphery was slightly yellowish. The Nile blue reactions were less satisfactory than those obtained with neutral red. If the area of necrosis was very marked, the response of the dye to the free hydrogen ion was shown by a definite blue color; but with moderate necrosis the color change often was insufficient to be satisfactory.

In order to minimize the formation of asphyxial organic acids during the time required to cut the sections and place them in contact with the indicators, the following method was used: Guinea pigs to which chloroform had been given two days previously for a period of four hours were again anesthetized with chloroform; and while unconscious the livers were removed. Sections were cut very quickly with a Valentine knife, washed in distilled water, and immersed in the dye on a slide. The whole process was sometimes done in less than one minute and always in less than two minutes. The neutral red was used as 1 per cent aqueous solutions; alcoholic phenylated Nile blue was used in different concentrations, but generally of 1 or 2 per cent strength.

In their work on the survival formation of lactic acid in amphibian muscle, Fletcher and Hopkins[10] have demonstrated that sarcolactic acid formation in frog muscles attains its maximum only after the lapse of hours, but in a much shorter time (thirty minutes) when the muscles, after removal from the frogs, are treated with chloroform vapor. In harmony with what is generally known concerning the action of narcotics during life there is no doubt that in chloroform poisoning the asphyxial formation of lactic acid is great. The question has therefore to be met whether the indicator changes described in the preceding paragraph are actually greater than might be explained by the presence of much asphyxial organic acid. This objection to interpreting the dye reaction as indicative of the presence of an inorganic acid in the necrotic areas is recognized and has not been removed. In fact when pieces of excised normal liver are kept for six to seven hours at 37° C. in order to insure their maximal content of survival acid, such tissues react to neutral red with about the same intensity as fresh chloroform livers. It is clear from this that the cell itself has the power to produce enough acid during life to effect the indicator changes described. The experiments are merely recorded in conjunction with other observations.

For the determination of an excess of chlorine ion in the necrotic areas sections of liver were cut in the same way as above and handled in general according to the method used by Macallum and Menten.[11] They were placed in N/10 silver nitrate, containing 1.5 per cent nitric acid, and kept in this bath, protected from the light, for from twelve to twenty-four hours. They were then mounted

[10] Fletcher, W. M., and Hopkins, F. G., Lactic Acid in Amphibian Muscle, *Jour. Physiol.*, 1906–07, xxxv, 247.

[11] Macallum, A. B., and Menten, M. L., On the Distribution of Chlorides in Nerve Cells and Fibres, *Proc. Roy. Soc., Series B*, 1906, lxxvii, 165.

in 50 per cent glycerol and exposed to the sunlight until the maximum effect was produced. The most pronounced blackening always occurred in the central necrotic portions of the lobules. The peripheries of the lobules were light brown instead of black. In control sections of normal livers the darkening amounted to only a brownish discoloration resembling the tint of the periphery of the lobule in a chloroform liver. This discoloration has been extensively discussed by Macallum and Menten, who consider that in the tissues it is a definite indication of the presence of chlorides.

Although an excess of free H^+ and Cl^- ions in the necrotic areas of the liver was clearly shown, nevertheless this finding alone cannot be interpreted as in any way indicative of the presence of free hydrochloric acid. The possibility of other sources of hydrogen ion has already been mentioned. Recently Fischer[12] has shown that protein gels (*i. e.,* fibrin) retain increased amounts of chlorides under the influence of acid. The excess of Cl^- ion in the necrotic areas, therefore, may represent only a greater accumulation or retention of neutral chlorides instead of hydrochloric acid.

As bearing on this point, however, it is of interest that the excretion of neutral inorganic chlorides in the urine is increased after the administration of chloroform, as has been shown by Zeller[13] and Kast.[14] Since hydrochloric acid formed in the tissues would doubtless be in part neutralized by metals in alkaline combination, this observation harmonizes with the present theory. Here also one must consider the possibility that an increased output of chlorides in the urine following administrations of chloroform may be due not so much to a splitting of the drug as to anomalies of metabolism and excretion set up in the process of narcosis. However, after the administration of iodoform there is, according to Mulzer,[15] an excretion in the urine of inorganic iodine indicative of a 60 per cent splitting of the iodoform. This could scarcely come from the tissues; and by analogy it would seem probable that the increased chloride excretion following chloroform administrations is due to

[12] Fischer, M. H., Relation between Chlorid Retention, Edema and "Acidos's," *Jour. Am. Med. Assn.*, 1915, lxiv, 325.

[13] Zeller, A., Ueber die Schicksale des Jodoforms und Chloroforms im Organismus, *Ztschr. f. physiol. Chem.*, 1883–84, viii, 70.

[14] Kast, A., Ueber Beziehungen der Chlorausscheidung zum Gesammtstoffwechsel, *Ztschr. f. physiol. Chem.*, 1888, xii, 267.

[15] Mulzer, P., Ueber das Verhalten des Jodoforms im Thierkörper, *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 446.

decomposition of this drug. Moreover, after narcosis with chloral hydrate the urinary chlorides show no such increase as with chloroform.

Necrosis-Producing Power of Other Chlorine Substitution Products of Methane.

A study of other chlorine substitution products of methane should be of interest from the standpoints of determining (1) whether they all have the power of producing central liver necrosis, and (2) whether this property is proportional to the number of molecules of hydrochloric acid which could theoretically be derived from them. If, starting with methane, we should outline a series of its various chlorine substitution products, we might expect, according to the theory, that those which could give the largest amounts of hydrochloric acid in their breakdown would manifest the strongest tendency to produce the necrosis and other changes. Thus *a priori* we might expect the series to run in this order:

$$CH_3Cl < CH_2Cl_2 < CHCl_3 < CCl_4,$$

if all of these substances were equally broken down in the body. Experiments were carried out to determine this point. The last three of the series (*viz.*, CH_2Cl_2, $CHCl_3$, CCl_4) were selected as sufficient to test the tenability of this hypothesis. Reason for the assumption that tetrachlormethane might have greater necrosis-producing power than chloroform, and this in turn than dichlormethane lies in the fact that, in the ultimate breakdown of these substances outside of the body in the presence of water, four molecules of hydrochloric acid can be obtained from one of tetrachlormethane, three from chloroform, and two from dichlormethane. Thus Goldschmidt[16] has found the saponification of tetrachlormethane at 250° C. to take place as follows:

$$CCl_4 + H_2O \rightleftharpoons COCl_2 + 2HCl$$

$$COCl_2 + H_2O \rightleftharpoons CO_2 + 2HCl$$

[16] Goldschmidt, H., cited by Meyer, V., and Jacobson, P., Lehrbuch der organischen Chemie, 2d edition, Leipzig, 1907, i, pt. 2, 23.

The well known reactions by which three molecules of hydrochloric acid can be derived from chloroform, by simple oxidation in the presence of water, have already been discussed on pages 49 and 50. Concerning the transformation of dichlormethane, André[17] has shown that in five hours at 180° C. 73 per cent is decomposed as follows:

$$2CH_2Cl_2 + 2H_2O \rightleftharpoons 3HCl + CH_2O_2 + CH_3Cl$$

$$CH_3Cl + H_2O \rightleftharpoons HCl + CH_3OH$$

Thus from one molecule of dichlormethane two molecules of hydrochloric acid may be formed.

When these various substances were administered to animals by inhalation, it was found that not only did all three possess the power of producing central necrosis of the liver, but that this power was shown in greatest degree by tetrachlormethane, notwithstanding its higher boiling point, and least by dichlormethane, the most volatile of the three. In all respects the toxicity of tetrachlormethane was greatest, and that of dichlormethane least. A comparison of the minimum fatal doses of the three substances when given intravenously to rabbits again bore out the same relationship as by the inhalation method. The following table shows the minimum fatal doses:

Substance.	Minimum fatal doses per kilo of rabbit.	Minimum fatal doses expressed in gram-molecular concentrations.
CCl_4	0.053 gm.	0.000344
$CHCl_3$	0.085 gm.	0.000711
CH_2Cl_2	0.147 gm.	0.00161

The quantities injected were shaken up with enough water to make one cubic centimeter.

In other words, both the general toxicity of these substances as well as their power to produce the extensive morphological changes paralleled the amounts of hydrochloric acid which they can give in their respective breakdowns outside of the body. The toxicity of tetrachlormethane was so great that if it were administered by inhalation for two hours the animal almost invariably died within twenty-four hours. At autopsy numerous hemorrhages were pres-

[17] André, G., Action de l'eau et de l'ammoniaque sur le chlorure de méthylène, *Compt. rend. Acad. d. sc.*, 1886. cii, 1474.

ent with extensive visceral edema, fatty changes, and beginning central liver necrosis. In a few instances the abdomen contained a large amount of free, unclotted blood. It was only by giving the tetrachloride for about one hour or less that an animal could be expected to survive long enough to develop a well marked typical liver necrosis. As has been said above concerning chloroform, the most extensive necrosis is seen about two days after the administration of tetrachlormethane. A two hour narcosis with dichlormethane is usually not sufficient to induce an outspoken necrosis of the liver. A comparison of the narcotic properties showed that dichlormethane is less powerful than chloroform. With tetrachlormethane it was difficult to produce a quiet narcosis analogous to that accompanying the use of chloroform. More or less severe muscular spasms, particularly of the extremities, occurred intermittently. This fact has already been noted by von Ley.[18]

The experiments were conducted as follows: Each of a series of three guinea pigs of approximately the same weight was placed under a bell jar, opened at the top to admit air. Into each jar was dropped enough of one of the three substances to induce narcosis, which was then carefully maintained at as nearly as possible the same depth and for the same length of time. Seven sets of animals were used; and the duration of the narcosis was varied from one and one-half to four hours. A typical protocol follows.

Guinea pigs A, B, and C, weighing respectively 550, 530, and 505 gm., were each put under a bell jar, as described above. To A was given dichlormethane; to B, chloroform; and to C, tetrachlormethane. These substances were administered for two hours. On the morning of the second day C was moribund; then all three were killed by a sudden, overwhelming dose of chloroform. At autopsy C (tetrachlormethane) showed several pulmonary and subperitoneal hemorrhages; the liver was large and fatty; and the kidneys were swollen and gray. B (chloroform) showed no marked gross changes except a large, fatty liver. A (dichlormethane) had a moderate accumulation of fat in its liver; but otherwise there were no gross changes of importance. Microscopically the liver of C (tetrachlormethane) showed definite areas of necrosis which involved nearly the whole of the lobule in each case, but which nevertheless had apparently begun at the central portion of the lobule. This necrotic area of each lobule contained cells with fragmented nuclei; and there was a tendency for the whole area to stain intensely with eosin. There were no parenchymatous cells evident which contained normal-looking nuclei except at the periphery of the lobule. There was a large amount of infiltrated fat, as shown both by staining with Sudan III and by the presence of many fat vacuoles in sections prepared and stained in the ordinary way with hematoxylin and eosin. The cells

[18] von Ley, Inaugural Dissertation, Strassburg, 1889.

of the kidney were swollen and granular, and fat vacuoles could be made out here and there. Guinea pig B (chloroform) microscopically showed changes which differed from those of C only in degree. The liver contained areas of well marked central necrosis, which, however, involved only about one-fourth to one-third of the lobule. A (dichlormethane) showed no definite areas of liver necrosis at all comparable to the other guinea pigs. But about the central veins there were occasional necrotic cells and a conspicuous accumulation of fat. The typical necrosis, however, such as is seen after chloroform, was obtained with dichlormethane when it was administered to a guinea pig for from four to six hours.

The Inhibiting Effect of Alkali.

If the theory is correct that chloroform liver necrosis is an effect chiefly of acid, then it might be expected that the administration of an alkali simultaneously with the chloroform would inhibit, if not actually prevent, its occurrence. Such was found to be the case. When sodium carbonate was given intravenously in a proper concentration in a hypertonic solution of sodium chloride, the liver necrosis was either entirely prevented or greatly inhibited. It was of course difficult to know in any given case how much alkali to administer, as it was not possible to estimate how much acid was being formed. Moreover, it was obviously desirable to avoid an excess of alkali, since this in turn will give rise to some of the serious effects of an excess of acid, such as swelling of protein colloids, etc., as shown by Fischer.[19] The amount of alkali given in each experiment was therefore decided empirically; and consequently the degree of inhibition of the necrosis was subject to wide variations in the different experiments. In only one instance, however, and that in the first experiment, was there a failure to observe a definite diminution of necrosis when alkali in hypertonic sodium chloride solution was administered. In this one instance the dose of alkali was excessive (17 grams of $Na_2CO_3 \cdot 10H_2O$ in 1,700 cubic centimeters of 1.4 per cent sodium chloride solution to a dog weighing twenty-one kilos, intravenously). Because of the onset, on the following day, of extreme thirst and a severe hemorrhagic nephritis, with passing of urine thick with blood, it was felt that clearly an overdose of the solution had been given. In all of six subsequent experiments, in which a much smaller dose was given, there was less

[19] Fischer, M. H., Oedema, a Study of the Physiology and the Pathology of Water Absorption by the Living Organism, New York, 1910.

necrosis than in the control animals which did not receive alkali. In one case no necrosis at all occurred. Another interesting fact was that in all of these six experiments the alkali animals seemed less toxic, and at autopsy other changes characteristic of chloroform poisoning, as well as the liver necrosis, were less conspicuous than in the control animals. This beneficial effect of the alkali was particularly striking in the kidneys. In the control animals, which received no alkali, these organs were always enormously swollen and weighed much more than those of the alkali animals. The effect of the alkaline hypertonic salt solution in inhibiting the swelling of these organs, tends to support Fischer's views on the nature and origin of edema. In only three of the experiments was the amount of visible fat in the alkali livers conspicuously less than in the controls. In none of the experiments was there noted any particular influence of the alkali either in strengthening or weakening the narcotic power of chloroform.

The alkaline solution used was that employed by Fischer in his work on edema; *viz.,* distilled water 1,000 cubic centimeters; $Na_2CO_3 \cdot 10H_2O$, 10 grams; sodium chloride, 14 grams. The method of conducting the experiments was as follows: The animals were always run in pairs; and the duration of the anesthesia was the same for both animals, as was also the depth of the narcosis, as nearly as could be determined. To one animal was given the alkaline solution and to the other was given the same quantity of a 0.85 per cent sodium chloride solution. Two sets of guinea pigs and four pairs of dogs were used, in all twelve animals, exclusive of the first pair already mentioned, in which clearly an excessive dose of alkali was given. The solutions were always injected into the blood stream. With the guinea pigs the injections were made by means of a syringe into the heart; but with the dogs, they were given by means of a cannula into either the saphenous or femoral vein. The solutions were always warmed to body temperature previous to being injected; and the amounts were varied in different experiments. In general, the injections were made shortly after the animals had lost consciousness, and they were given slowly. Two days after the administration of the chloroform the animals were killed with chloroform and examined. Pieces of the various tissues were fixed in Zenker's fluid and 10 per cent formalin and were stained both with hematoxylin and eosin and with Sudan III for fat. Typical protocols follow.

Experiment 3.—Two adult dogs. A weighed 4.5 kilos; B, 5 kilos. Both dogs were given chloroform by inhalation for four and one-half hours. The chloroform used was Mallinckrodt's, "Purified for Anesthesia." As soon as both dogs had lost consciousness cannulas were inserted into the saphenous vein; and into A were injected 150 cc. of the alkaline solution already described, and into B, 150 cc. of 0.85 per cent sodium chloride solution. Both solutions were

injected slowly over a space of one hour. Both dogs were still in a condition of deep narcosis when they were returned to their cages at the close of the experiment. On the next day Dog A (alkali) was lively and playful; but Dog B (control) was exceedingly drowsy and difficult to arouse. Water and meat were allowed freely. On the second day after the experiment both dogs were killed with chloroform and examined. The difference between the two dogs was very striking. Dog B (the control) had a very fatty, yellowish liver. There was a large subperitoneal hemorrhage on the liver; and there were numerous ecchymoses on the parietal peritoneum. The kidneys were swollen and gray in appearance; and together they weighed 68 gm. Dog A (alkali), on the contrary, showed no hemorrhages. There was little, if any, less fat in its liver than in B's, but the kidneys were practically normal in appearance and weighed only 43 gm. This marked difference in weight between the kidneys of the two animals is particularly striking since the weight of the dogs before the experiment was practically the same. Of still greater interest, however, is the difference in the microscopical findings in the two animals. The liver of B (control) shows areas of necrosis so extensive that practically the entire lobule is involved. There is little more than a fringe of cells about the periphery which have not lost their nuclei. The necrotic areas stain deeply with eosin; and the only nuclei that can be distinguished are those of the capillaries and not of the parenchymatous cells. There are numerous fat vacuoles, especially in the peripheral portions of the lobules. The liver of A (alkali), however, shows practically no necrosis. Only an occasional necrotic cell about the central veins is evident. The columns of cells stand out plainly, and their nuclei appear to be unchanged. There is a moderate amount of fat accumulation about the central veins. These differences are well shown in the accompanying illustrations (Figs. 1 to 4). The kidneys show also rather a marked difference, as might be expected from the striking difference in weight. The epithelium of the convoluted tubules in B's (control) kidney is swollen and granular, and it contains numerous fat vacuoles. There are occasional casts. A's (alkali) kidneys show no striking changes. The changes in the other organs were relatively slight in both dogs.

Experiment 4.—June 3, 1914. Two adult dogs: A, weighing 2.7 kilos; B, 2.5 kilos. Both dogs were given chloroform by inhalation for four and one-half hours. As soon as consciousness was lost A was given 80 cc. of the alkaline solution into the saphenous vein; and B was given the same amount of 0.85 sodium chloride solution. Two days later both dogs were killed with chloroform and examined. Both livers were very fatty, but microscopically B (control) showed much more necrosis than A. The pictures were very similar to those described in the previous experiment. The kidneys here again showed a striking difference. Those of B (control) weighed 35 gm., and those of A (alkali) only 26 gm., although A was the larger dog.

In the two experiments on guinea pigs the method consisted of putting two guinea pigs of approximately the same weight under a large bell jar and dropping chloroform into it through an opening at the top. In this way it was assured that both guinea pigs were breathing air with the same concentration of chloroform. After losing consciousness the alkaline solution was injected into the heart of one of the guinea pigs; and the other guinea pig was given 0.85 per cent

sodium chloride solution in the same way. Both were then returned to the bell jar, and the anesthesia was continued.

Production of Typical Chloroform Liver by Iodoform and Bromoform.

Iodoform (CHI_3) and bromoform ($CHBr_3$) are so similar to chloroform ($CHCl_3$) in chemical structure as to suggest that they might have a similar power to produce the characteristic morphological changes of chloroform poisoning. Here, however, we should of course be dealing with an effect of hydriodic acid and hydrobromic acid, respectively, instead of hydrochloric acid. As a matter of fact, not only do both of these substances induce lesions which are in every way identical with chloroform effects, but it is also possible to obtain some evidence that in each case the respective halogen acid is produced in the body. It has been known for some time that the administration of iodoform in large quantities is frequently followed by visceral fatty changes and multiple hemorrhages.[20] In our experiments we found, in addition to these changes, a definite central liver necrosis in every way comparable to that produced by chloroform. Bromoform was found to produce identical changes. Evidence that here also we were dealing with an effect of acid was obtained by finding that the necrotic areas in the liver reacted to neutral red in the same manner as has been described already for chloroform. Attempts to identify iodine ions in these necrotic lesions failed, but iodine was found in large quantities in the urine. It is not altogether surprising that we failed to find it in the tissues, since it doubtless occurs in small quantities at most; and, furthermore, because of its tendency to combine with fats and protein, probably nearly all of the iodine would be present in an organic, and hence non-ionic, form. The methods used for its identification will be described below. They were based on the standard means of identification of inorganic iodine by use of dilute sulphuric acid, sodium nitrite, and starch paste.

It is especially interesting that inorganic iodides may be found in large quantities in the urine after the administration of iodoform;

[20] Cushny, A. R., A Textbook of Pharmacology and Therapeutics, 3d edition, Philadelphia, 1903, 522. Meyer, H. H., and Gottlieb, R., Pharmacology, Clinical and Experimental, translated by Halsey, J. T., Philadelphia, 1914, 519.

for this necessarily implies the previous formation of hydriodic acid, at least somewhere in the body. The finding of iodides is an old observation. Thus Binz[21] in 1877 attributed the toxic action of iodoform to iodine, because of the presence of inorganic iodine in the urine. Hogyes,[22] Harnack and Gründler,[23] and Mulzer[24] have all concurred in this observation. Mulzer's study is especially interesting. He states that most of the urinary iodine is inorganic, in the form of alkali iodides and iodates. Of these, the iodides always appear first. Only about 60 per cent of the calculated iodine can be found in the urine. The rest is eliminated into the sweat, hair, and intestines. Different structures of the body vary in their power to transform iodoform into inorganic iodine compounds. Muscle and liver hash seem to be the most powerful; and the muscle hash is a little more powerful than the liver. These findings are all in harmony with the idea which is being developed in this article; *viz.*, that the decomposition of this group of drugs in the body is associated with the formation of the respective halogen acid, in this case hydriodic acid. Furthermore, if we regard this decomposition as essentially dissociation in the presence of water, as we did in the case of chloroform, then we are not surprised that it occurs in greatest quantity in the liver and muscle tissue where metabolic activity is greatest. The apparent discrepancy between Mulzer's and our own results, in that he found inorganic iodine in the liver where we failed to find it, is easily explained by the fact that he subjected relatively enormous quantities of iodoform to the action of the tissue hash and so obtained a recognizable amount of inorganic iodine which was not bound to protein. Even then his yields of iodine were small.

In our experiments with bromoform no attempt was made to find bromine ions. After demonstrating the identity of the morpholog-

[21] Binz, C., Ueber Jodoform und über Jodsäure, *Arch. f. exper. Path. u. Pharmakol.*, 1878, viii, 309.

[22] Hogyes E., Anmerkungen über die physiologische Wirkung des Jodoforms und über seine Umwandlung im Organismus, *Arch. f. exper. Path. u. Pharmakol.*, 1879, x, 228.

[23] Harnack, E., and Gründler, J., Ueber die Form der Jodausscheidung im Harn nach der Anwendung von Jodoform, *Berl. klin. Wchnschr.*, 1883, xx, 723.

[24] Mulzer, *loc. cit.*

ical findings obtained with chloroform, iodoform, and bromoform, and recognizing that chloroform and iodoform may form in the body hydrochloric acid and hydriodic acid, respectively, the parallelism with bromoform was so close that this detail was omitted as unessential. Typical protocols follow.

Experiment 5.—Adult dog, weighing 2.7 kilos. Injected daily subcutaneously with 2 gm. of iodoform (Merck), stirred up in 10 cc. of paraffin oil, for three days. On the third day when the injections were stopped, there was a strong reaction for inorganic iodine in the urine, as determined with starch paste. The dog had lost its appetite and had begun to look emaciated. On the sixth day (three days after stopping the injections) it was unable to stand, but, at intervals, while lying down, it would moan and howl feebly; and simultaneously there would occur frequent twitchings and cramp-like motions of the legs. At no time did the animal seem conscious of its surroundings. It refused meat and water; and between these times of activity it was drowsy and somewhat difficult to arouse. The strong reaction for iodine in the urine had persisted. The dog was killed with ether, but before death occurred a portion of the liver was removed and examined for free H^+ ions in the manner already described in connection with chloroform. The reactions to the indicators were less intense than with chloroform. Other liver sections were then treated for various periods for from one to thirty minutes with a little dilute sulphuric acid and sodium nitrite, later removed from this bath, and placed on slides. To the sections on the slide thin starch paste was added, and they were then examined to see if the necrotic portions of the lobules became blue. No reaction for iodine was obtained in this way. Then after merely removing enough of the liver for proper histological examination, the remainder was ground in a meat grinder and treated with sulphuric acid of about M/5 strength, and a little sodium nitrite. The fluid was then poured off and treated with starch paste. No reaction occurred. The anatomical changes noted in the dog corresponded in every way to those so characteristic of chloroform poisoning. There was a number of ecchymoses on the parietal peritoneum and pleura. The liver was large, very fatty, and slightly yellowish. It contained a small subcapsular hemorrhage on its upper aspect. The kidneys were large and swollen, and the site of marked cloudy swelling. Microscopically the changes again were identical with those which follow chloroform. The liver contained areas of central necrosis involving about one-fourth of the lobule. There was much fat accumulation in the cells that were not destroyed. The kidneys showed numerous casts; and the tubular epithelium was swollen and granular.

Experiment 6.—Guinea pig, weighing 560 gm. Given daily for three days subcutaneous injections of 0.2 gm. iodoform (Merck) in paraffin oil. On the fourth day the guinea pig was killed and examined. The anatomical changes were essentially the same as those described in the preceding experiment, but not developed to quite so great a degree.

Bromoform was found to be much more toxic than chloroform; so it was impossible to administer it for two hours to an animal and have any assurance that

it would live for forty-eight hours afterwards, and thus give time for the maximum development of the anatomical lesions. It was given by inhalation. In all, eight animals were used. A typical protocol is given.

Experiment 7.—A guinea pig weighing 672 gm. was placed under a bell jar and anesthetized by dropping bromoform through the opened top. It was kept in a state of deep narcosis for forty-five minutes and then returned to its cage and allowed to eat carrots. It remained lying on its side for nearly an hour after returning to its cage. Two days later it was killed by a blow on the head and examined. There were several small hemorrhages in the lungs. The liver was very fatty; and the kidneys were large and grayish. Microscopically the picture in the liver could not be distinguished from that of a case of chloroform poisoning. Around the central veins were small areas of necrotic cells without nuclei, constituting perhaps one-sixth or one-seventh of the lobule. There was a large amount of fat accumulation. Everywhere the changes were most conspicuous in the central portions of the lobules.

Liver Necrosis, Etc., as an Effect Common to Aliphatic Alkyl Halides.

The readiness with which central liver necrosis, fatty changes, hemorrhages, edema, etc., could be produced by methyl halogen compounds other than chloroform, immediately suggested the probability that ethyl and ethylene halides would react in the same way. Support for this idea was furnished also by the fact that these substances, like the methyl compounds, readily yield halogen acids outside the body. Niederist[25] has shown that at 100° C. ethyl iodide and water after fifteen hours yielded 98 per cent of calculated hydriodic acid, and that ethyl bromide after eighteen hours gave 94 per cent of calculated hydrobromic acid. Ethylene bromide after heating with water for fifty-two hours at 140° to 150° C. yielded 96 per cent of the theoretical hydrobromic acid.

$$\begin{array}{l} CH_2Br \\ | \\ CH_2Br \end{array} + \begin{array}{l} H_2O \\ \\ H_2O \end{array} \rightleftharpoons \begin{array}{l} CH_2OH \\ | \\ CH_2OH \end{array} + 2HBr$$

Butlerow[26] had previously found that, after heating ethyl chloride to 100° C. in a sealed tube for ninety-two hours, much hydrochloric acid and alcohol were present.

The following halogen substitution products of ethane have been

[25] Niederist, G., Ueber die Einwirkung von Wasser auf die Haloïdverbindungen der Alkoholradicale, *Ann. d. Chem.*, 1877, clxxxvi, 388.

[26] Butlerow, cited by Niederist, *loc. cit.*

tried in this work: ethyl chloride, ethyl bromide, ethyl iodide, and ethylene bromide. Not only do all these substances produce morphological changes indistinguishable from those following chloroform, but also it is possible to obtain evidence that the respective halogen acid is liberated in each case. This evidence is found in the fact that the neutral salts of the respective halogen acids have been found in the urine after the administration of ethyl bromide and iodide. That an acid effect is again concerned is shown by the fact that when sections of the liver are treated with neutral red, according to the method already given, the necrotic portions show a high hydrogen ion content. Although apparently no measurements have been made of neutral chlorides in the urine after the administration of ethyl chloride, an increase might nevertheless be expected by analogy with the findings after the use of ethyl bromide and iodide. Dreser[27] found inorganic bromine in the urine, both of man and experimental animals, after inhalations of ethyl bromide. Inorganic iodine has been found in the liver of a guinea pig after the use of ethyl iodide by Loeb.[28] The essential morphological changes under discussion in this article have been observed by previous workers to follow the use of ethyl chloride and ethyl bromide. These have been rather extensively studied by Haslebacher,[29] who also found that the urine became strongly acid and then contained large amounts of casts and albumen, an observation which, in the case of ethyl bromide, had previously been made by Regli.[30]

In our experiments it was found that when given by inhalation the relative power of producing the morphological changes as well as the general toxicity of these substances was greatest in the case of the iodide and least with the chloride. The most toxic of all was ethylene bromide. This was so toxic that a narcosis of fifteen minutes' duration or longer was invariably followed within forty-eight hours by death. In each instance Kahlbaum's preparations were used. Typical protocols follow.

[27] Dreser, H., Zur Pharmakologie des Bromäthyls, *Arch. f. exper. Path. u. Pharmakol.*, 1895, xxxvi, 285.

[28] Loeb, O., Die Jodverteilung nach Einfuhr verschiedener Jodverbindungen, *Arch. f. exper. Path. u. Pharmakol.*, 1906, lvi, 320.

[29] Haslebacher, A., Experimentelle Beobachtungen über die Nachwirkungen bei der Bromaethyl- und Chloraethylnarkose, Inaugural Dissertation, Bern, 1901.

[30] Regli, cited by Haslebacher, *loc. cit.*

Ethyl Bromide.—Adult guinea pig given ethyl bromide by inhalation for eighty minutes. Found dead about thirty hours after the administration. At autopsy the liver was fatty, and microscopically contained definite areas of central necrosis. There were extensive hemorrhages in the lungs. The kidneys were swollen and pale.

Ethyl Iodide.—Experiment performed as above, except that inhalation of ethyl iodide was continued for only forty-five minutes. The guinea pig was found dead twenty hours later. The liver was fatty, and microscopically there were areas of beginning central lobular necrosis. The kidneys were pale and apparently edematous. There were large hemorrhages in the lungs.

Ethyl Chloride.—After an inhalation of ethyl chloride for two hours, the guinea pig was killed two days later. It showed a moderately fatty liver with beginning central necrosis evident microscopically. There were no large hemorrhages in the lungs, and the kidneys were less pale and swollen.

Ethylene Bromide.—An adult guinea pig was given ethylene bromide by inhalation for twenty minutes. It was found dead forty hours later. Autopsy showed 2 or 3 cc. of a slightly blood stained fluid in the abdominal cavity. The liver was large and very fatty and showed beginning central necrosis. The kidneys were large and pale and the lungs had several large hemorrhages.

Lesions Produced by Chloral Hydrate Are Relatively Insignificant.

If the general theory is correct that the severe morphological changes induced by chloroform are due largely to hydrochloric acid liberated by its chemical dissociation in the tissues rather than merely to the fact that it contains three chlorine atoms, then we should expect that another narcotic substance, which would not yield hydrochloric acid in the body although it contained the same number of chlorine atoms, would not produce these changes. Chloral hydrate is a suitable substance with which to investigate this point, because it is eliminated almost completely as urochloralic (trichlorethylglucuronic) acid, and therefore is not capable of yielding appreciable amounts of hydrochloric acid. Only a very small portion of it is decomposed, with a resulting increase in urinary chlorides.[31] Experiments showed that guinea pigs, which had been profoundly narcotized with chloral hydrate for from fifteen to twenty hours, failed to show any liver necrosis, hemorrhages, or extensive edema. Only a very slight accumulation of fat occurred in the liver. This was demonstrable usually only by microscopic examination of sections stained for fat. Whipple[32] has stated that he also failed to produce liver necrosis with this agent.

[31] Meyer and Gottlieb, *loc. cit.*, p. 89.

[32] Personal communication.

These results seem to afford striking confirmation of the idea that the essential factor in the production of these severe lesions by alkyl halides is the halogen acid formed by decomposition rather than merely the halogen content of the molecule. A representative protocol follows.

Dec. 19. Three adult guinea pigs were used: A, weighing 615 gm.; B, 665 gm.; and C, 485 gm. At 12.30 each was given subcutaneously 0.2 gm. of chloral hydrate dissolved in 2 cc. of water. Fifteen minutes later Guinea Pigs A and B were lying on their sides, but still responsive to stimulation. At 3.00 p. m. there had still been no deep narcosis. Each guinea pig therefore was given 0.3 gm. At 4.30 each was given another injection of 0.1 gm. At 4.45 all three guinea pigs were in deep narcosis.

Dec. 20, 11.30 a. m. Each guinea pig was injected with 0.4 gm. An hour later all were in deep narcosis and were still so at 4.30 p. m.

Dec. 21. Guinea Pig B was found dead in the morning. Autopsy showed reddish purple liver with no appreciable fat accumulation and no noteworthy changes elsewhere. Microscopically there was found no appreciable accumulation of fat in the liver, no necrosis, and no marked changes in any of the viscera. At 11.30 a. m. Guinea Pigs A and C were given 0.5 gm. of chloral hydrate as before. At noon both animals were in deep narcosis. At 9.00 p. m. C died; A was still in profound narcosis. Autopsy on C showed slight fatty liver, but no hemorrhages or marked edema anywhere. Microscopically there was no necrosis in the liver.

Dec. 22. Guinea Pig A was found dead at 7.00 a. m. Aside from moderate postmortem decomposition there were no striking changes. Sections of the liver showed no central necrosis.

GENERAL DISCUSSION.

It is a striking fact that certain narcotic agents readily induce marked morphological changes, the most conspicuous of which are central necrosis of the liver lobules, fat infiltration, and a tendency to hemorrhage and edema. This property is particularly evident in those agents whose chemical structure places them in the group of alkyl halides. That this property is not necessarily connected with their ability to induce narcosis is shown by the fact that other narcotic agents (*e. g.*, ether and chloral hydrate), which do not belong to this general chemical group, fail to induce tissue changes which are at all commensurate with those following the administration of the alkyl halides. Moreover, that the mere presence of halogen atoms in the molecule is not the responsible factor is demonstrated by the fact that chloral hydrate ($CCl_3—CH<\begin{matrix}OH\\OH\end{matrix}$), which, like chloroform

($CHCl_3$), possesses three chlorine atoms, produces relatively insignificant morphological effects. Some other factor must therefore be responsible. Evidence has been submitted to show that an important factor is probably the halogen acid (hydrochloric, hydrobromic, or hydriodic acid) which is formed by chemical dissociation of the alkyl halides within the body. That these substances form their respective halogen acids in the body is shown by the occurrence in large quantity of the neutral salts of these acids in the urine. In this respect they differ from chloral hydrate, which is excreted mainly as urochloralic acid, and of which therefore only a small portion is decomposed to give neutral chlorides.

The idea of placing the chief responsibility for these severe tissue effects upon the halogen acids formed in the tissues is based upon a number of experimental findings. Pictures practically identical with that of late chloroform poisoning have been produced simply by the administration of hydrochloric acid in suitable concentrations, the only essential difference being that with the injection of the acid the liver necrosis was peripheral rather than central. That these substances form their respective halogen acids within the body is shown by the appearance of their neutral salts in the urine. Alkali, in suitable concentration and combined with hypertonic saline, prevented the liver necrosis and greatly inhibited the other tissue changes. In the series CH_2Cl_2, $CHCl_3$, and CCl_4, the tetrachloride was the most powerful and the bichloride the least, in their ability to induce the morphological changes. This comparison parallels the respective amounts of hydrochloric acid which these substances can yield in their breakdown outside the body; that is, CCl_4 can give four molecules of HCl; $CHCl_3$, three, and CH_2Cl_2, two. Of the ethyl compounds, the iodide was most toxic, the bromide less, and the chloride least of all. This relationship agrees with their relative chemical reactivities outside the body. The question of how the acid is formed will not be discussed extensively in this paper. Nef,[33] who has extensively investigated the nature of the chemical reactions of the alkyl halides, has submitted a large amount of evidence to show that the halogen acid is dissociated off, leaving a methylene

[33] Nef, J. U., Dissociationsvorgänge bei den Alkyläthern der Salpetersäure, der Schwefelsäure und der Halogenwasserstoffsäuren, *Ann. d. Chem.*, 1899, cccix, 126.

or bivalent carbon residue. In the case of chloroform the type of dissociation, according to him, is:

$$CHCl_3 \rightleftarrows CCl_2 + HCl;$$

and in the case of ethyl chloride it is:

$$C_2H_5Cl \rightleftarrows CH_3CH + HCl.$$

He has likewise produced evidence to show that in general the iodides are more dissociated than the bromides, and these in turn more than the chlorides. Our finding, therefore, that ethyl iodide produces the tissue changes more readily than the bromide and this in turn more readily than the chloride, conforms to Nef's idea of the readiness with which these substances can form respectively hydriodic, hydrobromic, and hydrochloric acids. The applicability of Nef's conceptions of dissociation and dynamic chemical equilibrium to problems of intermediate metabolism has already been under study for a number of years in this laboratory by Woodyatt, with particular reference to the chemical phenomena of diabetes.[34]

The tendency to ascribe the anatomical changes of chloroform to the production of phosgene ($COCl_2$), as has been done by Müller,[35] is probably an inadequate explanation, since this substance would almost certainly be quickly hydrolyzed to $2HCl$ and CO_2 in the body, and again we should be dealing with HCl as an important factor. Binz[36] has considered the liberation of the halogen itself (as molec-

[34] Woodyatt, R. T., Studies on the Theory of Diabetes. I. Sarcolactic Acid in Diabetic Muscle, *Jour. Biol. Chem.*, 1913, xiv, 441; Greer, J. R., Witzemann, E. J., and Woodyatt, R. T., Studies on the Theory of Diabetes. II. Glycid and Acetole in the Normal and Phlorhizinized Animal, *ibid.*, 1913–14, xvi, 455; Sansum, W. D., and Woodyatt, R. T., Studies on the Theory of Diabetes. III. Glycolic Aldehyde in Phlorhizinized Dogs, *ibid.*, 1914, xvii, 521; Woodyatt, R. T., Studies on the Theory of Diabetes. IV. The Parallelism between the Effects of the Pancreas and Those of Metallic Hydroxides on Sugars, *ibid.*, 1915, xx, 129; Sansum, W. D., and Woodyatt, R. T., Studies on the Theory of Diabetes. V. A Study of Narcotic Drugs in Phlorhizin Diabetes, *ibid.*, 1915, xxi, 1. Wells, H. G., Chemical Pathology, 2d edition, Philadelphia, 1914, 573.

[35] Müller, R., Ueber die Einwirkung des Phosgens auf den menschlichen und thierischen Körper, *Ztschr. f. exper. Path. u. Therap.*, 1911, ix, 103.

[36] Binz, C., *loc. cit.*

ular halogen) to be the chief toxic factor in all these drugs. There is, however, but little evidence in favor of such a conception; for at least outside the body most of the facts, as Nef has shown, point to a dissociation of a type to yield halogen acid instead of molecular halogen. Our experiments tend to show the existence of a similar type of dissociation within the body.

Fischler[37] has sought an explanation for the chloroform changes in an associated fat necrosis from injury to the pancreas which he noted in a number of dogs. This idea was based on the fact that central necrosis was produced in Eck fistula dogs without the use of chloroform but after intraperitoneal injections of trypsin and hydrazin sulphate, and after severe crushing of the pancreas. He considers some albumen-splitting substance, whose nature he does not discuss, as the responsible factor. Obviously such a suggestion does not explain the production of the changes. It is not surprising that such drastic measures resulted in severe morphological changes, of which one was central necrosis of the liver. It is interesting, however, that degenerative changes most conspicuous in the central part of the lobule (including even well marked necrosis), sometimes followed simply the establishment of the Eck fistula without other experimental procedures. In one experiment, in which he also ligated the hepatic artery, the central necrosis was marked.

Wells[38] has expressed the view that the changes in late chloroform poisoning arise because, although the oxidizing enzymes are suppressed, the autolytic enzymes are left free to digest the cell. There is no reasonable doubt that oxidations, as well as many other metabolic activities, are altered by chloroform and these other substances under discussion. It is likewise true that in many respects there is a similarity between the production of chloroform liver necrosis and the self-disintegration of tissue *in vitro* which is called autolysis. But the difficulty in such an interpretation lies in an inability to gain a definite conception of the nature of an autolytic enzyme and to distinguish its effects from those of acids formed in the tissues.

[37] Fischler, F., Ueber das Wesen der zentralen Läppchennekrose in der Leber und über die Rolle des Chloroforms bei dem sogenannten Narkosenspättod, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxvi, 553.

[38] Wells, *Arch. Int. Med., loc. cit.*

Particular care has been exercised all through this article to state that the halogen acids are suggested to be important factors rather than the only factors involved. Other acids must play a part; and possibly even other substances than acids are concerned. Since chloroform, like other narcotic agents, induces a severe tissue asphyxia, we are compelled to assume the presence of various organic acids, notably lactic acid; and lactic acid, as has been already shown by Fischer and others, is capable of producing extensive tissue alterations. The comparatively slight tissue changes which follow the use of ether and chloral hydrate, for reasons already stated, cannot be due to the liberation of a halogen acid in the tissues; but they may perhaps be attributable chiefly to the tissue asphyxia and resultant weak acid formation which they induce. Nor can a halogen acid be a responsible factor in poisoning with phosphorus, which produces morphological changes similar to those of chloroform, except that in the liver the most extensive alterations are at the periphery instead of the center of the lobule. The mechanism involved here is not clear. Cell asphyxia, however, without doubt occurs; and it is well known that lactic acid is formed in relatively large amounts,[39] which in itself could play an important part. In addition, the possibility should be considered of the formation in the tissue and action of some of the phosphoric acids which are known to occur so easily in the oxidation of phosphorus *in vitro*. As yet there is no direct evidence to support this view. Doubtless also other factors are more or less involved which concern physical alterations in the cell induced directly by the action of the phosphorus.

No detailed discussion will be taken up here concerning the question of whether these morphological changes (necrosis, fat infiltration, hemorrhages, and edema) are to be regarded as primarily acid effects or primarily asphyxial effects. It is of great interest that they can be readily obtained merely by the administration of such an acid as hydrochloric acid. But it is equally true that the administration of an acid also leads to the production of asphyxia. We are, therefore, confronted by the facts that acids give rise to asphyxia, and asphyxia in turn gives rise to acid production. It cannot be assumed that the changes are exclusively acid effects.

[39] Lusk, G., The Elements of the Science of Nutrition, 2d edition, Philadelphia and London, 1909, 304.

In this connection the location of the necrosis in the central part of the lobule is of interest; for this is the region which is farthest removed from the oxygen supply and from compensatory influences carried in by the blood. It is, therefore, the part where, in general asphyxial conditions, asphyxial acids (lactic, etc.) would be formed in largest quantity. This is strikingly borne out in Fischler's experiments which showed that interference with blood supply, by ligation of the hepatic artery and production of Eck fistula, was followed by degenerative changes which were always most marked in the centers of the lobules. In the case of chloroform poisoning we might suppose that the central location of the necrosis is due to the fact that in that region we have the greatest total acid formed (*i. e.,* asphyxial acids in addition to hydrochloric acid). It is also farther removed from the neutralizing effect of alkalis brought in by the blood than is the periphery of the lobule.

In a previous article[40] it has been shown that newly born pups, which are relatively immune to the production of late chloroform poisoning, owe their resistance to their rich supply of glycogen. The nature of this protective action of glycogen is not clear. It is possible that the observation of Bechhold and Ziegler,[41] that glucose retards the diffusion of sodium chloride and some other substances in protein gels, bears on this phenomenon. In some work, as yet unpublished, we have observed that the presence of glucose in gelatin and agar gels markedly retards the diffusion of hydrochloric and other acids through them. Fischer and Sykes[42] have recently shown that glucose and other sugars inhibit the swelling of a colloid, like fibrin, in water.

SUMMARY.

The central lobular necrosis in the liver, which has been regarded by some writers as characteristic of late chloroform poisoning, has been produced experimentally with a number of other drugs. It

[40] Graham, E. A., The Resistance of Pups to Late Chloroform Poisoning in Its Relation to Liver Glycogen, *Jour. Exper. Med.,* 1915, xxi, 185.

[41] Bechhold, H., and Ziegler, J., Die Beeinflussbarkeit der Diffusion in Gallerten, *Ztschr. f. physikal. Chem.,* 1906, lvi, 105.

[42] Fischer, M. H., and Sykes, A., Ueber den Einfluss einiger Nichtelektrolyte auf die Quellung von Protein, *Kolloid-Ztschr.,* 1914, xiv, 215.

is, therefore, in no sense peculiar to chloroform poisoning. Substances which have been shown to produce a morphological picture indistinguishable from that of late chloroform poisoning are: (a) dichlor- and tetrachlormethane, (b) tribrom- and triiodomethane, (c) monochlor-, monobrom-, and monoiodoethane, also the dibromethane; that is, in general, the halogen substituted aliphatic hydrocarbons containing one or two carbon atoms. Presumably similar results might be obtained with the higher members of the same series.

The mechanism by which chloroform produces its characteristic tissue changes must accordingly be considered as a group reaction. Outside the body the similarities between the chemical behavior of different members of this group have been correlated by Nef on the basis of the type of dissociation which these substances undergo and the differences in their behavior on the basis of the differences of the degree to which such dissociations occur. According to the work of Nef, the group of substances under discussion has the property of dissociating to yield a halogen acid and an unsaturated alkylidene rest. Thus with chloroform the type of dissociation may be expressed thus:

$$CHCl_3 \rightleftharpoons CCl_2 + HCl.$$

In this paper the view is developed that the changes characteristic of late poisonings with the above named group, namely edema, multiple hemorrhages, fat infiltration, and necrosis are ascribable (1) to acids and (2) to the fact that the amount of acid formed parallels the chemical dissociability of the drug outside of the body.

Favoring the view that acid is responsible for the changes are the following observations.

1. All the characteristic features of late chloroform poisoning have been produced merely by the administration of hydrochloric acid, except, however, for a different distribution of the liver necrosis.

2. The areas of central necrosis produced in the liver by the various substances under discussion give an acid reaction to neutral red.

3. Sodium carbonate in a hypertonic sodium chloride solution markedly inhibits the production of the lesions.

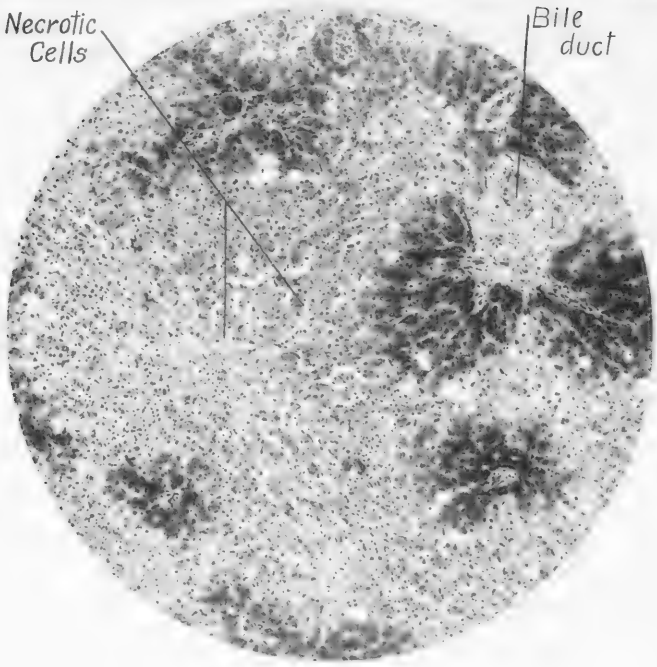

FIG. 1.

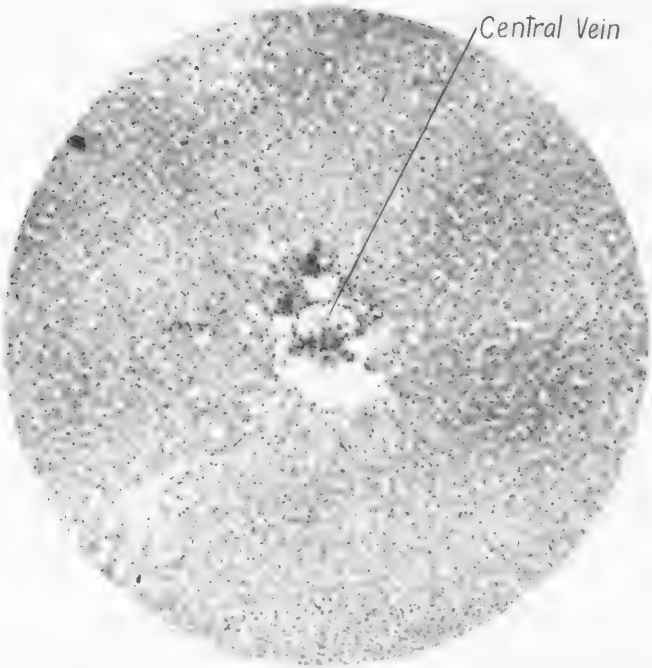

FIG. 2.

(Graham: Late Poisoning with Chloroform.)

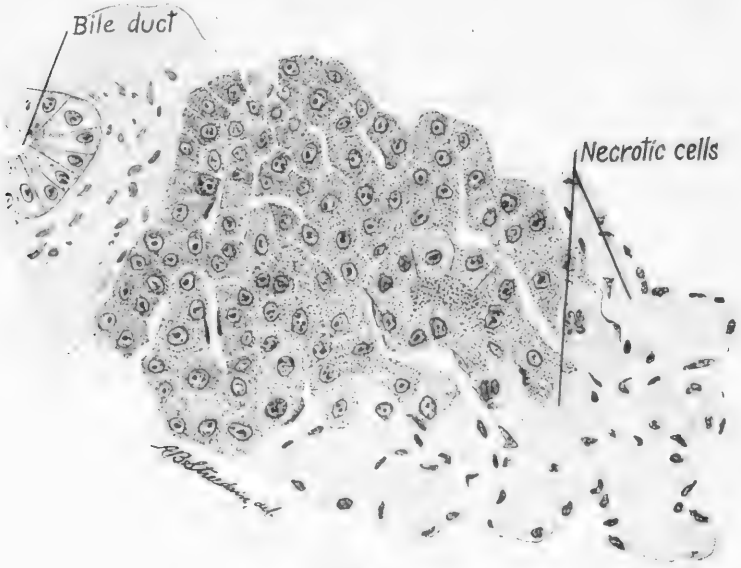

FIG. 3.

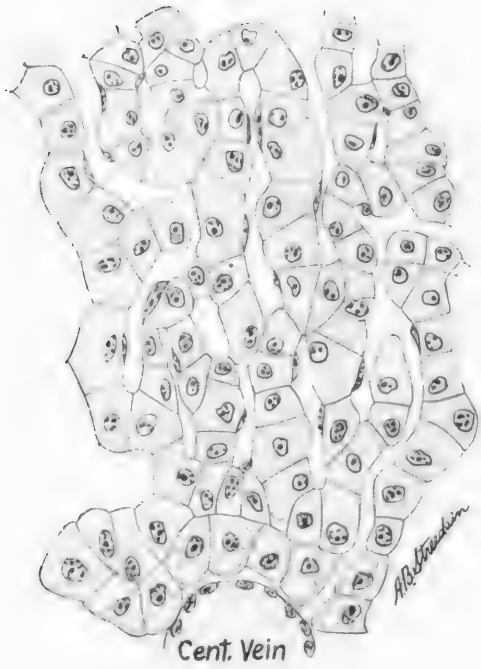

FIG. 4.

(Graham: Late Poisoning with Chloroform.)

In favor of the view that the respective halogen acids play an important part are the following.

1. After the administration of some of these drugs there has been noted an increase of the neutral salts of the halogen acids in the urine, a fact which indicates that the corresponding halogen acids must have been formed somewhere in the body.

2. The necrosis-producing powers of dichlormethane, chloroform, and tetrachlormethane parallel the amounts of hydrochloric acid which these substances theoretically can yield in their breakdown outside of the body. Likewise, the power to produce tissue changes exhibited by the ethyl compounds varies directly with the ease with which they form their respective halogen acids *in vitro*.

3. Ether and chloral hydrate which do not yield halogen acid in their breakdown in the body likewise also do not produce necrosis. They induce only edema and fat infiltration to a less marked degree.

The suggestion is made that the halogen acid (hydrochloric, hydrobromic, or hydriodic acid), directly liberated in the process of dissociation, may be the important factor which makes the tissue changes seen in poisoning with chloroform and other alkyl halides so different from those following the administration of narcotic drugs of a different type.

EXPLANATION OF PLATES.

Plate 4.

Fig. 1. Typical central liver necrosis produced by chloroform anesthesia for four and one-half hours in Dog B of Experiment 3 (page 61). Nearly all the lobule is affected. Microphotograph. × 83.

Fig. 2. Inhibition of necrosis by the intravenous injection of 150 cc. of Fischer's alkaline, hypertonic salt solution as shown in Dog A of Experiment 3 (page 61) which had received chloroform for the same length of time as Dog B (Fig. 1). There is practically no necrosis. Only a slight amount of fat accumulation in the cells about the central vein has occurred. Microphotograph. × 83.

Plate 5.

Fig. 3. High power drawing of liver shown in Fig. 1. The necrotic cells in the central portion of the lobule stain intensely with eosin. The nuclei of the parenchymatous cells have disappeared; and only those of the capillaries are evident. × 356.

Fig. 4. High power drawing of the liver shown in Fig. 2. There is no appreciable necrosis; and only a moderate accumulation of fat has occurred in the parenchymatous cells around the central vein. × 356.

THE FORM OF THE EPITHELIAL CELLS IN CULTURES OF FROG SKIN, AND ITS RELATION TO THE CONSISTENCY OF THE MEDIUM.

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Plates 6 to 21.

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INTRODUCTION.

In a previous publication (1) the author has shown that the polygonal epithelial cells of frog skin assume a fusiform or thread-like shape ("spindle cells" and "thread-like columnar cells") shortly after they have wandered out into the medium. In this form they closely resemble connective tissue cells.

Champy (2) found in the case of the explanted kidney that both connective tissue and epithelial cells gradually took on an indefinite character, and he observed the same phenomenon in the explanted parotis, submaxillaries, and thyroids. It is of especial importance to note that the indefinite tissue of these organs is absolutely identical, and that, for example, the tissue derived from the kidney cannot be distinguished from that from the parotis. He calls this phenomenon "dedifferentiation," and attributes it to loss of function. It seems to us, however, that but little is gained by this explanation, for we possess no information regarding the functions of these organs in the embryonic stage and are unaware whether, for instance, the parotis or submaxillary functionates at all in the fetal organism. The point to be emphasized is rather the fact that organs as different as the kidney, salivary glands, and thyroids develop identical tissues as soon as the same chemicophysical conditions are brought to bear on the respective organs. This result signifies that the factors responsible for this equalization are pres-

ent in the culture medium. Thus, in view of the fact that we can modify at will the character of the medium, we are supplied with a definite means of further analyzing these phenomena.

It stands to reason that the above mentioned changes occurring in the cells are the result not of one factor, but of a number of different chemical and physical factors. In this paper, however, we intend to confine ourselves to the description of but one of these factors and to show in what way the consistency of the medium influences the form of the cell.

It is an easy matter to vary the firmness of the medium, which consisted of frog plasma and frog muscle extract obtained from the same species (*Rana pipiens*) that supplied the fragments of skin. The plasma of all individuals of the same species does not coagulate uniformly well, and it was soon apparent that this fact greatly influences the form which is subsequently assumed by the cells. Furthermore, by the addition of chicken plasma the frog medium can usually be made to gain a semi-firm consistency, and by the combined addition of chicken plasma and embryonic chicken extract the medium becomes very firm.

In previous experiments (1) we carried out prolonged observations on cultures that were cultivated in a pure frog medium (frog plasma + frog muscle extract), and found that the rim of tissue which formed around the explanted fragment of skin began by assuming the form of a compact membrane consisting of polygonal cells. Next the cells situated at the edge of the membrane began to project out into the medium, at the same time taking on an elongated spindle-shaped form (spindle cells). Many of these cells became detached from the membrane and wandered out into the plasma. By this means the edge of the membrane assumed a ragged appearance. Finally spaces occurred in the membrane which were bridged over by very elongated cells, often thread-like in appearance (thread-like columnar cells). As a result of successive processes the entire membrane gradually became divided up into long cells of the connective tissue type which strayed out into the medium.

It has now been shown that this change in the form of the tissue membrane and of its cells results from the gradual softening of the medium, and we are thus able, by the employment of suitable media, to produce artificially the individual stages of this process, accompanied by the characteristic form of the cells. The shape of the cells can be maintained permanently polygonal or fusiform, according as the medium selected is firm or soft.

As in our previous experiments, the material chosen was obtained from the dorsal skin of the adult leopard frog (*Rana pipiens*), which was cultivated in small fragments (approximately two square millimeters). The following are the results obtained with 179 cultures, and they are in accordance with the findings and records of the 480 cultures of our previous work.

EXPERIMENTAL.

Experiments with a Firm Medium.—We shall first describe the experiments made with a firm medium. A medium was considered firm when, in the process of changing, the culture could be cut out of the old medium enclosed in a firm block of medium. The firmness of these blocks was so great that they failed to bend over the edges of the cataract knife, even when they were considerably broader than the knife itself. We usually obtained such a medium by adding to two drops of frog plasma (F.Pl.)[1] one drop of frog muscle extract (F.E.), one drop of chicken plasma (Ch.Pl.), and one drop of embryonic chicken extract (Ch.E.).

Fig. 1 shows a culture (B 174) in the firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.); photographed about twenty hours after explantation. A considerable rim of tissue has formed around the old fragment. It consists of strictly polygonal cells, and its line of demarcation towards the medium is uniform or only slightly irregular. The cells are closely pressed together. A vertical section of these cultures shows that the rim of tissue usually consists of from three to four layers of cells at the center, and of two layers at the periphery, and that the cells are polyhedral in shape, as shown in Fig. 2. In Fig. 3 are seen from above some of these polyhedral cells taken from a culture fixed *in toto* and stained with Giemsa (B 169); it was prepared in the same medium as culture B 174 in Fig. 1. Thus viewed we clearly see the polyhedral shape of the cells.

Fig. 4 (Culture B 114) represents a stained culture after a six days' sojourn in a firm medium. The culture was prepared on Dec. 15 and the following day showed but a relatively small rim of cells. On Dec. 17 the fragment of skin, together with the cell membrane, was placed in a fresh medium. On Dec. 19 the rim of cells had increased in size while remaining compact. It is noteworthy that no migration of cells had taken place. On the same day the old medium, which had remained completely firm, was exchanged for a fresh medium, into which was transferred the entire membrane of tissue. On the morning of Dec. 31 the medium showed signs of liquefying on one side. One protuberance into the medium had already formed, as well as a small cavity, which latter, however, was subsequently destroyed by fixation (to the left of the figure). After six days the culture was fixed. The membrane consisted exclusively of polygonal cells (Fig. 4), with the exception of that portion where the medium had begun to liquefy. Fig. 5 represents a part of the epithelial rim of Culture B 114 mag-

[1] We shall abbreviate as follows: frog plasma, F.Pl.; frog muscle extract, F.E.; chicken plasma, Ch.Pl.; chicken embryonic extract, Ch.E.

nified 1,000 times. The polygonal shape of the cells is distinctly seen. It should be noted that the tissue membrane of this culture (as also of all others subjected to the same conditions) maintained its compact form for six days without becoming loosened up, and that even the outermost cells of the rim, shown in Fig. 5, remained polyhedral in shape.

Fig. 6 was taken from a living culture (B 103) ten days after explantation. The culture was started on Sept. 12, 1914, and at first showed but slight signs of growth. The epithelium did not attain any considerable dimensions until Dec. 13, at which time it was quite thick. On Dec. 14 the medium was changed for the third time, whereupon it was found to be of exceedingly firm consistency. Thus it was possible to transfer the large tissue membrane without injury into the new medium. On Dec. 15 the rim of tissue was not as compact as in similar cultures which had been maintained under the same conditions. When the medium was changed on the same day it was also found to be less firm than that of the other cultures. Nevertheless, it was possible to cut out a block sufficiently firm to permit of the transfer of the entire rim of cells into the new medium. The cells of the outermost edges, however, which were the result of the growth from Dec. 14 to 15, tended to become massed together. On the 16th additional increase in the size of the cell membrane could be seen and new cells had been added. No migration of cells had taken place, however, and the membrane was quite compact. On Dec. 17 the medium was again changed. On Dec. 19 enlargement of the membrane could again be seen in certain sections, but the form of the membrane remained uniform and was unindented. No cell migration had taken place. At this stage the culture was photographed (Fig. 6). To the left of the figure can plainly be seen the thin layers of cells which sprouted out on the day preceding that on which the photograph was taken. They are separated from the remaining tissue by a lighter band of tissue. No detached cells can be seen around the culture. The latter, therefore, maintained for ten days consecutively a compact rim of tissue, which grew almost continuously and failed to undergo loosening up. The cells remained permanently polygonal in shape.

As long as the medium remained firm the same conditions were observed in all the other cultures subjected to the above treatment.

Experiments with a Medium of Semi-Firm Consistency.—A medium of semi-firm consistency was supplied by the addition of chicken plasma to the frog plasma and frog muscle extract (2 drops of F.Pl. + 1 drop of F.E. + 1 drop of Ch.Pl.). With a medium of this consistency it is still possible to remove a clean-cut block from the drop of plasma, but these blocks are so soft that if they extend beyond the edge of the knife their sides double over and adhere to the under surface of the knife. In order, therefore, to transfer into a fresh medium large blocks of this consistency with the enclosed culture every precaution must be used to avert this doubling over.

Moreover, it should be stated that owing to the extreme fluctuations in the consistency of the above mixture (F.Pl. + F.E. + Ch.Pl.) the results here obtained were by no means as uniform as those secured with the mixture composed of F.Pl. + F.E. + Ch.Pl. + Ch.E. Sometimes, for instance, a very firm medium is produced, at other times a soft one, such as results from the use of a pure

frog medium. But it is easy in each case to ascertain the degree of firmness. Here, however, we shall mention only the behavior of the cultures kept in a semi-firm medium; for their behavior when in a firm or soft chicken plasma mixture (F.Pl. + F.E. + Ch.Pl.) is the same as when kept in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.) or in a pure frog medium (F.Pl. + F.E.), respectively.

Fig. 7 represents a culture about twenty hours old (B 86), cultivated in a mixture of F.Pl. + F.E. + Ch.Pl. The rather broad rim of cells is seen to be very dissimilar from the tissue membranes obtained in a firm medium. The central zone of cells cannot be clearly made out in the photograph, owing to too intensive staining. Nearly all the peripheral cells have assumed a spindle-shaped form. At several places sharp projections of cells can be seen jutting out into the medium, and at their extremities the typical fusiform cells are beginning to stray out individually into the medium. This process of migration is seen to be specially active at the projection seen in the right-hand upper corner of the photograph, as a result of which a small space has been formed in the membrane. The rim of tissue has consequently assumed an irregular, serrated appearance, and the medium in the neighborhood of the culture is beginning to fill up with detached fusiform cells that have strayed out, as can be clearly discerned in the figure.

The above characteristics are seen still more plainly in a three day old culture (B 7), which is shown under higher magnification in Fig. 8. The edges of the membrane project into the medium on all sides in the form of sharp tongues, the cells of which are elongated examples of the spindle form. An enormous number of cells have wandered out into the medium, and consequent upon this migration and upon the advance of the border cells, it is seen that behind the edge spaces of varying size are being formed in the membrane. These spaces, however, contain no liquid.

Figs. 9 a and 9 b are drawings of living cultures made without a camera lucida. They represent the process involved in the projecting out of the cells at the edge of the membrane and their ultimate detachment and migration (page 86).

It is possible to carry on for a prolonged period of time observations with chicken plasma cultures in the same manner as previously described in connection with the cultures grown in a firm medium, and furthermore to transfer them practically uninjured into fresh media. Cultures kept in a plasma of semi-firm consistency maintain permanently the characteristics described above, in contradistinction to those maintained in a firm medium, in which the cell membrane is permanently compact and the form of the cells unvaryingly polygonal, while the latter usually refrain from wandering off into the medium. Culture B 78 was observed for a period of twenty-two days. Not until the sixth day had a fairly large rim of epithelium formed; later, however, it became greatly reduced in dimensions, owing in part to considerable cell migration, and in part to losses incidental to change of medium. After fourteen days, as a result of this, only a small rim of cells remained, and some of the peripheral cells had become spherical in form, owing to insufficient nourishment, a circumstance which is apt to occur in any medium. On the fifteenth day, however, the peripheral cells

recovered, and began to form a new thin membrane, the edges of which showed the usual sharp indentations, and consisted of greatly elongated cells. Two subsequent transfers again occasioned a reduction of the membrane, and this was followed by renewed active growth of the fusiform cells accompanied by the usual migration of cells. The same process was repeated after another change of medium, at which stage the culture was photographed. Fig. 10 represents Culture B 78 twenty-one days after explantation. The central parts of the membrane consist chiefly of polygonal cells; the edges, however, show sharp and multiform indentations, and their cells, even those remote from the edge, are spindle-shaped. Cell migration can likewise be clearly seen.

After twenty-two days Culture B 78 was fixed and stained with Giemsa, in which condition it appears in Fig. 11. The membrane of cells is seen to be even more loosened up than on the preceding day, and long tissue processes, consisting of elongated spindle-shaped cells, are seen to project into the medium. The migration of cells has assumed exceptionally large proportions; the detached cells are for the most part fusiform in shape, but some are round.

Fig. 12 shows individual spindle-shaped cells, drawn under a high magnification. In Fig. 12 a we see the transformation of typical polygonal cells of the membrane into fusiform cells. Figs. 12 b and c represent typical examples of the isolated fusiform cells.

Experiments with a Soft Medium.—The phenomena which we have observed in the medium of semi-firm consistency occupy a position midway between those occurring in the firm and in the soft media. We shall now consider the latter. A soft medium is obtained by employing a pure frog medium (F.Pl. + F.E.). It is impossible to cut out little square blocks of this medium; on the contrary, these blocks invariably assume a spherical form, irrespective of the shape in which they have been cut out, and they furthermore have a tendency to adhere to the knife. Soft media can be drawn out in threads (isolation of the fibrin); they contain much liquid which is accumulated in the minute vacuoles in the network formed by the fibrin. These media rapidly succumb to complete liquefaction, whereupon a number of particular phenomena take place, which we must consider in connection with the rest, as they usually occur coincidently.

Fig. 13 was photographed from a living culture (B 119), about twenty hours after explantation of the fragment. We are unable to determine whether in this culture a "secondary epithelial rim," as we termed in our first communication the compact rim of epithelium, preceded the loosening up stage, illustrated in this figure, as the former process may have developed over night. Nevertheless, in cultures consisting exclusively of frog medium the cells frequently begin to leave the fragment immediately in the form of a star-shaped membrane, with very ill defined edges; for these pure frog media usually become soft very soon, sometimes from the very outset. Fig. 13 gives a good impression of the condition of the cell membrane in a soft medium. Almost the entire rim of epithelium is drawn out in the form of long chains of cells and bands of tissue. Even the compact parts of the tissue membrane consist exclusively of elongated cells. On all sides large spaces are seen in the tissue membrane, lined by highly delicate, thread-like cells. These spaces contain no liquid, but soft medium. The whole form of the rim of tissue clearly indicates that the tissue is in a condition

of active movement, as can also clearly be seen in living cultures under the microscope. The chief characteristic of this particular culture is the enormous migration of cells. The entire drop of plasma is filled with wandering cells, which are partly fusiform in shape, partly round. In this culture whole fragments of tissue strayed out into the plasma. We were able to certify that they moved in a soft, not liquid substrate, by shaking the culture, a test invariably applied by us; for fragments floating in a liquid move from side to side, which did not occur in this case.

Fig. 14 shows Culture B 121 (stained with Giemsa), six days old, which was kept under parallel observation with the above mentioned Culture B 119 (Fig. 13). The culture was started on Dec. 15, and on Dec. 16 already possessed a fairly extensive star-shaped membrane of tissue, which projected out into the medium in long tissue processes, as is the rule in a soft frog medium culture. On Dec. 17 the medium was renewed, and on Dec. 19 the membrane of cells had attained considerable proportions. It was characterized by extremely long projections of tissue, as well as by bridges of thread-like columnar cells, and exceptionally active cell migration. The medium was renewed the same day, and on Dec. 21, six days after explantation, the culture was fixed, stained, and photographed (Fig. 14). The same characteristics can be noted as in Fig. 13, but more strongly emphasized.

Fig. 15 is a four day old culture in a pure frog medium photographed while living (B 117). The medium has partly liquefied and the resulting liquid has collected first of all in the holes in the tissue membrane caused by the migration of the peripheral cells. In such cases we are unable to decide whether these holes are primarily formed through the wandering out of the cells, as occurs in non-liquid cultures, or whether the membrane becomes torn by the vacuoles of liquefaction which are in process of being formed. The vacuoles, which at first are small, soon increase in size, the bridges of cells which separate them at the same time becoming thinner and thinner until at length they break away. By this means one or more larger vacuoles are formed, which in turn force the remaining part of the tissue to give way at the sides. Finally the cells are left in the form of a narrow wreath of cells encircling the large vacuole. In Fig. 15 can be seen the delicate, thread-like columnar cells which connect the ring of cells with the old fragment. At one place in this culture a single fragment of compact, but very thin tissue membrane yet remains; here the plasma had remained very firm, so much so that it was possible to cut out a block containing the membrane and transfer it into a new medium.

At this point it may be well to consider the behavior of the uppermost layers of epithelial cells. In Culture B 117 the same have become disconnected and are seen to lie over the old fragment and over a part of the cell membrane in the form of a thin membrane (Fig. 15) which possesses sharply defined edges while its cells have remained inactive. This phenomenon will be discussed in detail in a subsequent communication.

Cultures which are preserved in a pure frog medium, or in soft or liquid media generally, can only in rare cases, favored by every precaution, be maintained for any length of time as actively growing tissue membranes; usually the cells migrate in such large numbers that in a short time nothing remains of the

original membrane of cells. But it occasionally happens that a portion of the epithelium, instead of migrating, grows around the connective tissue either partially or completely, thus retaining its epithelial character. This particular phenomenon, however, which we have mentioned elsewhere (1), does not concern us here, and will be reserved for future consideration. It behooves us, rather, to follow the fate of certain cultures which when cultivated in a soft frog medium retained their membrane in a growing condition for a considerable length of time.

Culture B 77 was started on Nov. 28, and the tissue membrane took a fairly long time to develop. The records fail to show clearly at what time this took place. On Dec. 13, fifteen days after explantation, the membrane of cells still showed signs of growth at its edge, and was transferred on Dec. 14 to the new medium in an incomplete condition. On the following day, however, there were signs of renewed growth. On the same day the culture was again supplied with medium, and on Dec. 16 fresh growth of spindle-shaped cells was noted. At this stage the culture was photographed (Fig. 16), eighteen days after explantation. The cell membrane is seen to be exceedingly delicate and much thinner than that of a twenty-one day old culture grown in a medium of semi-firm consistency (Fig. 10). The tissue protuberances are much longer and narrower, and some of the cells are as fine as threads. The migration of cells is very active.

Similar conditions obtained in the case of Culture B 81, which was likewise started on Nov. 28 in pure frog medium. By Dec. 2 a narrow rim of epithelium with long protuberances and columns of thread-like cells had formed. On Dec. 13, one day after the medium had been changed for the eighth time, long fusiform cells still projected from the rim of membrane, and on Dec. 14 the membrane had again greatly increased in size. On Dec. 16, after two more changes of medium, the rim of tissue was still growing and projecting long processes into the medium. By Dec. 19 the entire membrane had become loosened up and an enormous number of bipolar fusiform and round cells could be seen in the plasma which had partially liquefied. The cells had strayed to the very limits of the drop of plasma. Fig. 17 shows the living culture photographed on the same day (twenty-one days after explantation). The spherical zone of liquefaction can be plainly seen, containing innumerable fusiform and round cells.

The behavior described above of the membranes of cells in connection with the old tissue fragment, is likewise characteristic of individual, isolated fragments of membrane when grown in daughter cultures. Fig. 18 shows an isolated fragment of membrane (Culture B 100 a) in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.), and Fig. 19 represents a fragment of a tissue membrane (Culture B 119 a) in a soft medium (F.Pl. + F.E.). The former was taken from Culture 100, the latter from Culture 119. Culture B 100 a produced two days after isolation a fairly thick membrane, consisting of polygonal cells, whereas in Culture B 119 a twenty hours after isolation the elongated fusiform cells had formed a connective tissue-like network of cells. The thread-like columnar cells had developed exceptionally well in this culture, and can be seen in great numbers in Fig. 19.

At this stage it might be asked whether the above mentioned variations which we have ascribed to differences in the consistency of the medium, should not rather be attributed to the chemical differences prevailing in the three kinds of medium, in view of the fact that a firm consistency was secured by the addition to the pure frog medium of chicken plasma and chicken embryonic extract, which thus at the same time enriched the culture by the addition of the specific chemical materials characteristic of the body juices of a foreign species.

The extended period of growth of the said cultures is a sufficient indication that these foreign substances exert no injurious effect on them. For reasons not yet known it occasionally occurs that in spite of the addition of these foreign substances, the medium yet fails to solidify but remains soft, and in such cases the form of the membrane and cells is seen to be identical with those observed in a pure frog medium. On the other hand, it occasionally happens that a pure frog medium or one to which chicken plasma alone has been added, remains unusually firm; the result in such cases will be the formation of great compact rims of tissue and polygonal cells, such as are usually seen only in a culture composed of F.Pl. + F.E. + Ch.Pl. + Ch.E. The following illustrations prove this point. Fig. 20 shows a culture which was kept for about twenty hours in a pure frog medium. The rim of tissue is uncommonly thick and consists chiefly of polygonal cells; only in the left-hand top corner have the cells begun to assume a spindle form. Fig. 21 is a two day old culture (B 13), which was kept in a mixture of F.Pl. + F.E. + Ch.Pl. In this case the medium was exceptionally firm, the epithelium as a result thereof exceedingly compact, and the cells polygonal in shape. Fig. 22, on the other hand, shows culture B 100 which was grown in a mixture of F.Pl. + F.E. + Ch.Pl. + Ch.E., and which for a period of ten days possessed an exceedingly compact rim of tissue, consisting of polygonal cells. Here cell migration failed to occur. On the tenth day the culture was transferred into a new medium, which in spite of being composed of the customary mixture failed to coagulate entirely. The membrane thereupon immediately assumed the form characteristic of one grown in a semi-firm medium; *viz.*, in a combination of F.Pl. + F.E. + Ch.Pl. At the same time the cells became fusiform in shape and began to migrate.

Consistency of the Medium and Form of the Cells.

We have shown that the shape of the cells is dependent upon the consistency of the surrounding medium. We have also shown that the fusiform and thread-like epithelial cells of the connective tissue type, described in a former publication (1), arise only in media of a semi-firm or soft consistency, whereas epithelial cells grown in a firm medium remain permanently polyhedral. These observations

may be considered as a distinct advance, as they furnish information regarding the causes governing the shape of the cells. Nevertheless, we can not yet arrive at a complete understanding of this important morphological phenomenon, for we are confronted by the question as to how and why the shape of the cells is affected by change in the consistency of the medium. Neither our own experiments nor those of others at present offer a satisfactory explanation; but it is important definitely to realize along what lines among the knowledge and facts at our disposal we should search for a solution of the problem.

Our point of departure is a two-fold phenomenon: first the increased cell migration in a soft medium; and second the difference in the shape of the cells consequent upon the varying firmness of the medium.

Burrows was the first to consider in an exhaustive manner the effect of the dilution of plasma on the growth of tissue. He found that the cells move more rapidly and actively in plasma which has been diluted with tissue juices than in undiluted plasma, and he attributes this fact chiefly to the reduction in the fibrin content of the medium, consequent upon this dilution (3), and secondly, to the reduced density of the medium (4). From his demonstrations, however, we fail to learn that by diluting the plasma the fibrin content was diminished, for, according to Burrows, upon pressing out the liquid portion of the medium a firm block of fibrin again remained, which although reduced in thickness had nevertheless preserved its consistency.

Be that as it may, the problem of a reduction in the content of fibrin is not involved in our method, for in the media which contained relatively the largest quantity of fibrin (F.Pl. + F.E. + Ch.Pl.) the migration of cells was considerable, whereas in the media which contained less fibrin but more muscle extract (F.Pl. + F.E. + Ch.Pl. + Ch.E.) no migration whatever took place. As a matter of fact the relation between the cell migration and fibrin content was exactly the reverse from what would have been expected according to Burrows. With respect to density, however, our findings agree with those of Burrows. The maximum density coincides with a complete lack of isolated migrating cells, whereas in a pure frog medium, *i. e.*, one possessing a minimum density, the cells become detached from the tissue membrane and stray out into the medium in enormous quantities.

The form of the cells is closely connected with their movement; both phenomena originate from the same cause. By careful investigation of the above facts it will remain evident that neither can be ascribed to "activity" on the part of the cells, but are purely passive phenomena. The cell is impelled to move, and it receives its form by the same processes which necessitate movement, for which reason these two phenomena must be studied in combination.

We shall consider the various forms of cells in succession, beginning with the fusiform cells at the phase of their evolution from the polygonal type. This can be done without difficulty by selecting specimens whose medium is undergoing a change from the firm to the soft stage. Here we at once remark that the spindle-shaped cells are invariably formed from the border cells which are surrounded on three sides by the soft medium; they never arise from the polyhedral cells situated in the more central parts of the membrane where the latter is three to four layers thick. The border cells, as well as all the other cells constituting the membrane, possess a clearly differentiated ecto- and endoplasm. The latter is relatively dense and foamy in structure, containing vacuoles, granulations, and the nucleus. The ectoplasm, on the other hand, is homogeneous, and only plainly visible when the light in the microscope is partly shut off (Figs. 9 and 9 a). The above facts can be clearly distinguished in preparations *in toto,* which have been fixed with formol and stained with Giemsa (Fig. 12). The transition from the polygonal to the fusiform shape of the cells is a gradual process. First of all the ectoplasm projects from the edge of the membrane into the medium in the form of one or more exceedingly delicate but broad processes. This results at first in the cell's assuming a flask-shaped form (Fig. 9 a Ia), and moving out into the medium. Many of the cells, however, do not become immediately disconnected upon starting this movement, but remain attached on one side to several of the back-lying cells of the membrane, which often results in the posterior end of the projecting cell becoming long drawn out (Fig. 12 a, Fig. 9 b Ia). As soon as this connection is severed the posterior side of the cell usually contracts immediately, sometimes also the anterior end (Fig. 9 a III, 9 b IIa), after which the ectoplasm gradually disperses into the medium again from the anterior end of the cell, the posterior end again becoming drawn out into a long projection (Fig. 9 a IV and Fig. 9 b III a). By means of these processes uni-, bi-, and tripolar fusiform cells are formed. This migration of the peripheral cells causes the cells situated nearer the center of the membrane to be brought into contact with the medium, whereupon they undergo the same changes as the cells which first migrated. From the circumstance that the migrating cells fail to become dissociated immediately from the remaining cells of the membrane the latter similarly become drawn out, and thus chains or triangular processes are formed composed of elongated cells.

If we base our conclusions on the fact that the dispersion of the peripheral cells, which initiates the change in form, as well as the

movements of the polyhedral cells, occurs only in comparatively soft, but never in firm media, the fact is thus primarily established that the consistency of the medium is highly instrumental in causing these phenomena. Thus, there must exist a definite relation between the consistency of the medium and the consistency of the cytoplasm in order that the movement of the cells may be initiated. Additional light is thrown on this problem by a consideration of the observations which led Burrows to the formulation of his well thought out theory of cellular movement; for our own experiments fail to account for the dissociation from the tissue of the first migrating cells and the subsequent temporary contraction of their posterior end.

Burrows has proved that the mechanical laws underlying the movements of the cell are identical with Jacques Loeb's tropisms. According to Burrows, cells which are surrounded by a medium containing a uniform distribution of metabolic products are invariably spherical in shape in consequence of uniform contraction of the cytoplasm on all sides of the periphery of the cell. A culture, however, which has been freshly supplied with medium, does not possess this uniform distribution of metabolic products, for these are found in greatest concentration in the proximity of the original fragment, and in least concentration near the edge of the drop of medium. Thus, only the posterior end of the cell, *viz.*, that adjoining the old fragment of tissue, will become contracted. By means of repeated contraction of the posterior end and flowing out of the anterior end the cell is induced to move away from the old fragment.

This brilliant theory which is based on a series of painstaking observations lacks, however, one point; it fails to explain the extension of the anterior end of the cell. Our observations, therefore, here serve as a supplement to those of Burrows and are as follows: The cell plasma in flowing out simply obeys the law of gravity, and this movement is initiated at the moment when the relation between the consistency of the medium and the consistency of the cell plasma has attained a certain value, not yet ascertained, brought about by the reduction of the firmness of the medium.

This explanation likewise accounts for the shape of the fusiform cells; that is, of the unipolar ones. But even the bipolar form assumed by the migrating cells might be explained thus. We have seen that the peripheral cells, the free ends of which flow out into the medium, remain connected with the back-lying cells for some time longer, causing a passive elongation of the posterior end to take

place. Later this connection is severed and the posterior end of the cell again becomes first contracted and then elongated. It may safely be assumed that the part that was in the first instance played by one of the cells of the inner rows is now assumed by a fibrin-thread or by other fragments of tissue lying in the medium, to which the posterior end of the cell adheres. Thus, contraction can only take place at the moment that the tension, which is threatening the rupture of the progressively thinner posterior end, has attained a certain maximum, and is added to the contracting force resulting from the products of metabolism. The cell will, therefore, be compelled to continue its advance, assuming at times a bipolar and at times a unipolar form.

From the above it is primarily evident that an isolated cell must be invariably accompanied by the occurrence of the fusiform shape, unless the conditions become changed in such a way as to cause the cell to become round instead (page 89). As the isolation of cells is possible only in relatively soft media (in accordance with the law of gravitation), we are unable to find examples of fusiform cells occurring in firm media. It is easy to account for the polyhedral form of the cells in a firm medium which are united to form a membrane and which, as in the case of the flattened form assumed by the cells attached to a firm base, must be a result of surface tension. If in addition the cells are made to adhere to a firm base they become flat instead of polygonal in shape. For this reason the cells situated over the connective tissue of the explanted fragment are always flat, whereas the cells which are distributed on all sides in the form of a compact membrane and are uniformly surrounded by the firm medium, take on a polygonal shape, even if they were originally flat, as happens in the case of the cells of the middle epithelial layers. The cell retains this polyhedral shape until the firmness of the medium is reduced to the point at which it is possible for the border cells to flow out.

Thus we see that the cells' adoption of the polyhedral and fusiform shapes is not an active process, and not confined to a definite class of cells; for the epithelial cells can also become passively fusiform, in which condition it is hard to distinguish them from connective tissue cells. There is no doubt that the thread-like columnar

cells are likewise passively transformed epithelial cells; this change can at any time be observed in living cultures. They originate through tension from the polyhedral cells and are always seen to occur at places where the membrane has become torn and holes are formed in the tissue membrane. This, however, only occurs in semi-firm and soft media. In both media the peripheral cells wander more rapidly than the other cells, because a greater portion of their surface is in contact with the medium. By this means continuous tension is exerted on the central parts of the membrane, and the outer parts are thus gradually disconnected from the inner. The cells which are slow in giving up their connection become exceedingly drawn out before breaking off, as occurs in the case of the posterior end of the fusiform cells, and they thus bridge over the holes which have been formed. To this must be added in soft media the formation of vacuoles, by which the same result is effected.

We must now endeavor to account for the form of the round cells, of which mention has repeatedly been made. Round cells are found in all the media employed. The liquid medium contains them to the exclusion of all other forms (Fig. 17) whereas they are but rarely found in a firm medium and only under special conditions. In media which have liquefied but a few hours after the preparation of the culture, membrane formation never occurs, but all the cells rapidly become converted to the round type, and the entire rim of cells, even if when originally cultivated in a firm medium it consisted of a membrane of tissue, is then seen to be composed of loose round cells, irrespective of the frequency of the change of medium. In many cases the fusiform cells, after wandering about for a long time in an isolated state, become surrounded with a zone of liquid and then likewise become round in shape.

The round cells (Fig. 23) are distinguished from the other varieties by the fact that their cytoplasm almost always assumes a purple color in cultures stained with Giemsa, which suggests a difference in the chemical constitution of the round cell. Many of them possess a normal nucleus, some a pycnotic one (Fig. 23 a), and others no nucleus at all. Even cultures which have undergone complete loosening up and are surrounded by a fluid medium are still seen to contain a considerable number of normal round cells. Thus, the round

cell seen in Fig. 23 c originated in a culture (B 81) which had undergone complete loosening up (Fig. 17).

It is seen that the round cells are yet alive from the circumstance that many of them resume the fusiform shape as soon as they are placed in an appropriate medium. This was seen to occur in living cultures observed under the above conditions. Moreover, it is also possible in living cultures to witness the conversion of fusiform into round cells. Fig. 24 shows a cell of a twenty-two day old culture in the process of transformation from the fusiform to the round shape; it is moving around in the medium. Here a dark, yellowish endoplasm, filled with vacuoles and granulations, can be distinguished from a light, homogeneous ectoplasm. The latter is in constant wave-like motion. The changes in shape are continuous and very rapid in comparison with those of the fusiform cells. This particular cell wandered about in every direction, alternately approaching and leaving the fragment, but never continuing for long in the same direction.

Burrows found that the cells assumed a round form when they were placed into a medium containing evenly distributed products of metabolism. Nevertheless, the facts appear to us to argue that in addition to the chemical constitution of the medium account should also be taken of the consistency of the latter, in view of the fact that in liquid media round cells only were seen, and that the spindle-shaped cells in the non-liquid media always become spherical when surrounded by a small quantity of liquid. It may be suggested that the relation between the chemical constitution of the medium and that of the plasma on the one hand, and the relation between the consistency of the medium and that of the plasma on the other hand are also instrumental in determining the form of the cell. The point whether the round cells are normal or degenerate must depend on the length of time that they have ceased movement. Round cells which have been stationary for a long period of time doubtless degenerate as a result of the progressively deteriorating quality of the surrounding medium consequent upon metabolism. These are the round cells in which the nucleus is either pycnotic or entirely absent. At all events, it would be erroneous to designate the round cell *per se* as a degenerate form.

In this section we have seen that the shape of the cell is not an

active manifestation on the part of the cell, nor even connected with the cellular functions or with the origin of the cell (connective tissue or epithelium). The form of the cell is in no way connected with the morphologic character possessed by it while in the organism, which it introduces into the culture; on the contrary, the flatly polygonal epithelial cell can be made to assume a polyhedral, fusiform, or round shape, if the relation between the chemical constitution and the consistency of the medium on the one hand, and of the cell plasma on the other, be varied accordingly. Certain forms, such as the thread-like form of the columnar cells and of the posterior end of the bipolar spindle-shaped cells, are produced directly through tension.

We wish to mention that the method used by us possesses many disadvantages, which, however, we intend to rectify in our later experiments. It is above all necessary to utilize a medium which is either less exposed to a spontaneous variation of its consistency, or one at least in which this factor can be strictly controlled. In our subsequent investigations we shall endeavor to utilize media, the consistency and firmness of which may be accurately expressed in mathematical terms. Owing to the fact that our investigations bearing on this point are not yet sufficiently advanced, they can not here be taken into consideration.

Form and Function of the Cells.

If in our work with cultures we are able to observe how through external agents a change of form is imposed upon the epithelial cells, rendering them very similar to the connective tissue cells, it is but reasonable that these observations should be borne in mind when we return to an examination of the cells in the organism itself.

Hitherto the relations existing in the organism have not been investigated from this point of view; on the contrary, the study of the shape of cells and organs has been conducted from a standpoint according to which they are considered as an expression of cellular activity and as a consequence of cellular function. Moreover, the study of these relations in the organism itself, with exact methods subject to the control of the observer, is a problem much more difficult to compass.

Nevertheless, we believe that by observations allied to those above described we shall be led to a clearer understanding of the morphological character of the cell than is possible by the adoption of other methods which proceed from the theory of functional adaptation.

The phenomena observed in the cultures likewise obtain in the epithelia in the organism. For example, frog skin epithelium consists of a basal layer of cuboidal cells and of several layers of flatly polygonal cells. The situation of the former is similar to that of our polyhedral membrane cells; they are enclosed between two comparatively firm media, the connective tissue and the more or less tense flat epithelial cells. It is, therefore, not surprising that they possess a similar form. It may be that the remaining flat cells of the epithelium owe their form to the tension there prevailing, a condition which can not be similarly produced in the cultures by artificial means. The flat cells of the cultures always originate at places where the cells lie between two media of different firmness; in such cases they appear to be pressed flat to the firmer of the two media by means of adhesive forces. Flat cells of this type either adhere to the coverglass (Harrison, Lambert, etc.), or, as observed in our own cultures, to the connective tissue. All the cells which remain attached to the connective tissue become flatly polygonal in shape. Nevertheless, in the organism cases are also seen to occur in which the epithelium is not under tension on all sides, but where the cells stray out freely while adhering to the connective tissue-like base. In such cases all the epithelial cells become flat in form, including those which were previously cuboidal in shape. This condition is always found in regenerating epithelial wounds (5). Here many epithelial cells also assume a spindle form, as they wander out from the edges of the wound into the wound scab, where they find similar conditions to those prevailing in the plasma medium. An abundant exudate in the wound, containing serum and fibrin, may furnish a medium more or less soft and conducing to this change. Leo Loeb (6) observed such spindle-shaped epithelial cells in the skin in process of regeneration in rabbits and guinea pigs.

The significance of these observations is apparent when they are compared with Champy's interpretation of his important observations made in connection with tissue cultures. Champy (2) states that the cells of some organs when

cultivated in blood plasma undergo certain changes which he calls "dedifferentiation." In cultures of the kidney, submaxillary, parotis, and thyroid from the fetal rabbit the cells of the glandular tubes soon begin to multiply actively through mitotic division. The minute canals sprouting from the cut tubules at first retain their epithelial character, but they immediately forfeit their character of gland cells. Finally, the basal membrane of these canals bursts and the epithelial cells mingle with the connective tissue cells of the interstitial tissue. At this stage they also lose their epithelial character and in conjunction with the connective tissue cells form a new tissue which is completely indefinite in character. Moreover, the interesting point in this connection is the fact that the indefinite tissue produced by all four organs is identical.

Such a process of "dedifferentiation," however, occurs only in organisms the cells of which undergo mitotic division. Therefore, it fails to occur in the organs of adult animals, which remain inactive outside of the organism. In the case of explants of cartilage, however, although mitotic division occurs, dedifferentiation fails to take place. The cells simply revert to a primitive condition, while at the same time they remain differentiated as cartilage cells.

Thus we find, as Champy himself realized, that for the "preservation of functional structure" function itself is by no means indispensable. For there are many organs the cells of which, failing to undergo mitotic division, preserve their so called "functional differentiation" when explanted. In this category we must include above all certain adult organs in which loss of functional structure would be expected to take place much earlier than in embryonal organs; that is, if the declarations of those who support the theory of functional adaptation be correct.[2] The cartilage, moreover, in spite of mitotic division, remains permanently cartilage, although of a primitive form, and in this case no atrophy from inactivity has occurred, as would have been a necessary presupposition in a case of functional adaptation. The case under consideration is a parallel of that of the transplanted salamander eyes. In these cases it was shown that although the animals were kept in the dark and had had their optic nerves cut, thus depriving the eyes of function and functional stimulus, these organs nevertheless failed to lose their functional structure (7). Thus far do the theories of Champy coincide with the established facts.

But he next concludes that the functional structure fails to arise in the newly formed cells owing to the culture's lack of functional stimulus. He bases his

[2] Roux' "functional period," which is supposed only to commence at an advanced, or at least, postembryonal stage.

theory on the following consideration. Before undergoing mitosis a cell first discards its differentiation. As the newly formed cells in the body are immediately subjected afresh to the influence of functional stimulus, they at once resume their functional structure; but in cultures which are devoid of all functional stimuli the reestablishment of the functional structure remains absent. Champy sets up this hypothesis immediately after observing that cartilage fails to dedifferentiate, although when cultivated its cells undergo mitotic division.

As a matter of fact the problem at issue in Champy's experiments must be looked for elsewhere. If every cell when about to undergo division renounces its functional structure and becomes embryonal, as has often been observed to be the case and as Champy himself admits, it is evident that the cells of the culture, even when exposed to functional stimuli, can never take on their original functional structure, as they continually proceed from division to division. It is therefore incorrect to assert that the absence of functional structure is to be ascribed to absence of function. The problematic point is rather the fact that the cells in the cultures never have a chance of developing. Were it possible for them to do so they would simply develop into their normal, inherited structures, among which are included the majority of characters which, according to Champy, are functional structures. That part of the structure, however, which owes nothing to the factor of inheritance is seen to arise passively in the cell, even in the absence of functional stimulus, as soon as any forces are brought to bear upon it (8).

We should like to recall the interesting experiments of Burrows in connection with heart muscle cells, as bearing on the question of the relation between development and differentiation in the cultivated cells. These heart muscle cells can be artificially reduced to a condition at which cell division takes place, at which stage they also undergo "dedifferentiation." If, however, the cell division is intercepted the cell is able to develop, and upon reaching the end of its development it begins to pulsate normally, independently of the operation of any specific functional stimuli.

It is, however, incomprehensible how, for instance, "lack of function" in the culture can be designated as the cause of the difference existing between the cultivated and the fetal parotic cells, in view of the fact that this lack of function exists in the body itself. For at the embryonic stage the parotid of the rabbit has not yet begun to functionate.

Finally, we must again revert to Champy's observations on the explanted cartilage.

Thoma (9) has repeatedly attempted to replace Roux' doctrine of functional adaptation by a new theory, which he designates as histomechanism and histochemistry. According to Thoma's histomechanical laws, cartilage can be formed from connective tissue only when the forces of pressure and tension have reached a certain maximum; as soon as they fall below this degree the cartilage is unable to subsist and reverts to connective tissue.

As has been recognized by Roux, this theory is fundamentally identical with the theory of functional adaptation; the difference is only one of name, as was shown in the case of the cartilage cultures. For it was seen that although the conditions of pressure prevailing in the plasma cultures differ essentially from those found in the body, the cartilage nevertheless continues to exist as such. Its cells by no means revert to the connective tissue type of cell, but they merely assume a more primitive character as a consequence of the mitotic divisions to which they are subjected. Here, therefore, the theory of functional stimulus is again at fault.

In conclusion we should like to call attention to another hypothesis of recent origin, by which it is sought to explain the variations in the morphological characters of the cultivated cells. We refer to Champy's so called theory of "inhibition" (10), a hypothesis no less obscure than that of functional adaptation.

Champy noticed that a tissue fails to dedifferentiate if it is explanted together with another "antagonistic" tissue, although when cultivated alone it rapidly undergoes dedifferentiation. When connective tissue and epithelium are cultivated together, the latter not only fails to dedifferentiate, but as in the cases of cicatrization of wounds, it grows around the connective tissue and remains a typical epithelium.

This observation of Champy's is accurate, and we have already reported similar findings in a previous communication (1). By the above described process we even succeeded in obtaining minute epithelial organisms, filled with connective tissue. But upon making an extensive examination of 600 cases we discovered that this result was but rarely obtained, and only in cases where the connective tissue was considerably firmer than the surrounding medium. In such cases the cells are pressed on to the connective tissue by means of simple

adhesion, just as under certain conditions the cells in a firm medium may be pressed on to the still firmer cover-glass. The fact that this results in their assuming a flat polygonal shape can certainly not be attributed to any inhibiting influences, but simply to the force by which they are being pressed against the base. Moreover, the statement that the epithelial cells always dedifferentiate as soon as they wander out into the medium is incorrect, for in firm media in which the epithelial cells migrate into the medium, they remain united in the form of an epithelial membrane and also preserve the typical form of the epithelial cell. The fact that the cells which in the body were flat in shape under these circumstances also assume a polyhedral form is to be ascribed to purely physical influences. Champy himself has shown that the cells remain united in the form of a membrane; but he believes that these cells are not epithelial, because the mitotic spindles are situated parallel instead of vertical to the plane of extension of the membrane. This latter fact we have observed to be correct (Fig. 5); nevertheless, it would be a mistake to assert that such a phenomenon never occurs in true epithelia, nor in the organism itself. Fig. 25 shows the regenerating epithelium in the tail of a tadpole (*Rana pipiens*). Such a regenerating epithelium shows many examples of mitosis in which the spindle is parallel to the surface of the cutis; we have one such example in our figure. No one would venture to state that regenerating epithelium is not true epithelium, and for that reason we must consider Champy's judgment to be inaccurate. The epithelium loses its inherent characteristics only when the cells are placed in a soft or semi-firm medium (according to our relative nomenclature), where the cytoplasm begins to disperse and the cells, now fusiform in shape, begin to separate, owing to the movements which are being initiated. Then only does it become hard to distinguish them from the ordinary connective tissue type of the cultures. Thus, the above processes are by no means caused by the suspension of some inhibiting influence, but simply through the physical changes in and around the cell.

The technique of tissue cultivation permits us to study the elementary parts of organs (the cells) with great exactitude. The more we are enabled, by means of our modern methods of technique, to pursue this detailed study, the more are we able to dispense with

the theories of "inhibiting influences" and "functional stimuli." By means of the above experiments in which we have shown that the form of the cell is dependent on the relation existing between the consistency of the medium and that of the cell plasma, we believe to have added some small contribution toward the attainment of this end. Changes in the consistency of these two substances, such as can be introduced into the culture by means of chemical procedures, result in a variation in the shape of the cell. Two additional factors of importance are, moreover, surface tension and adhesion, as was earlier shown by other investigators. But the effect produced by these latter must similarly depend upon the relative consistency of the medium and of the cell plasma. The next point of importance will be to establish the mathematical values of this relation, as applied to the various forms of cell. It is our intention to consider this aspect of the problem in subsequent investigations.

SUMMARY.

1. Fragments of skin from the leopard frog (*Rana pipiens*) were cultivated in media of varying consistency. A mixture of frog plasma, frog muscle extract, chicken plasma, and chicken embryo extract usually produced a very firm medium; a mixture of frog plasma, frog muscle extract, and chicken plasma, one less firm (semi-firm); and a mixture of frog plasma and frog muscle extract a medium of a consistency varying from soft to liquid.

2. (*a*) In a firm medium the cells which migrate into the medium are polyhedral (polygonal when seen from above) in form, which shape they retain permanently. They remain united in a compact membrane, the central parts of which consist of several (three to four) layers of cells. Migration of isolated cells into the medium does not take place.

(*b*) In the semi-firm media the cells situated at the edge of the membrane become fusiform in shape, gradually detach themselves from the membrane, and stray out individually into the medium. This causes the membrane to become loose in character, and to contain holes, while its edges at the same time become very irregular and send out pointed projections.

(*c*) In a soft medium the cells are fusiform or thread-like in shape. The migration of isolated cells is much more pronounced than in the semi-firm media, as a result of which the membrane undergoes constant and rapid loosening up. By this means whole portions of the membrane become detached and their separate parts are at first united by the thread-like columnar cells, which become drawn out in the form of long threads upon the separation of the individual sections of the membrane. The loosening up of the membrane is further assisted by liquefaction and the consequent formation of vacuoles; the latter process likewise results in the formation of thread-like columnar cells.

(*d*) Liquid media contain only round cells.

3. This serves to explain numerous internal processes of the organism, especially certain changes of form observed by Leo Loeb in transplantations of wound scabs and of skin; the conditions artificially produced by Leo Loeb must have effected a change in the consistency of the medium.

4. It has been shown that it is unnecessary, for a satisfactory explanation of the above findings, to have recourse to the theories of "functional stimulus" or "inhibiting influences," by means of which Champy wished to account for the variations in the morphological character of the cells.

I wish here to acknowledge my indebtedness to Mr. Louis Schmidt, for the care with which he has made the photographic reproductions of my cultures. Mr. Schmidt has succeeded in working out a new method for counteracting the destructive effects of the condensation of water which is apt to occur in the cultures when exposed to the rays of the arc light after having been previously kept at a low temperature (21° to 23° C.).

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EXPLANATION OF PLATES.

Plate 6.

Fig. 1. Frog skin, cultivated in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.); about twenty hours after explantation. A dense membrane of polygonal epithelial cells surrounds the old fragment. A small vacuole still exists on the right side only, which has caused some cells to become elongated. Culture B 174; Giemsa stain; magnification × 30.

Fig. 2. Frog skin, cultivated in a semi-firm medium (F.Pl. + F.E. + Ch.Pl.); three days after explantation. Part of the cell membrane, fairly remote from the border of the membrane, where the latter still consists of two to three layers of cells. As shown by this cross-section, they are cuboidal in shape; as shown in Fig. 8, seen from above, they are polygonal. Therefore their actual shape is polyhedral. Camera lucida drawing; Culture 7. Cross-sections stained with Giemsa solution after fixation in potassium bichromate, 70 per cent; glacial acetic acid, 20 per cent; formalin, 10 per cent; magnification, Leitz oc. 3; obj. 1/12, oil immersion; tube 170.

Fig. 3. Frog skin, cultivated in a firm medium (F.Pl. + F.E. + Ch.Pl. +

Ch.E.); about twenty hours after explantation. A piece of the border of the cell membrane, seen from above, where the cells have a polygonal shape. The spaces between the cells are artificially produced. Camera lucida drawing. Culture B 169. *In toto* preparation, stained with Giemsa solution after fixation in formalin 2 per cent; magnification, Leitz oc. 3; obj. 6; tube 160.

PLATE 7.

FIG. 4. Frog skin, cultivated in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.); six days after explantation. A dense membrane of polygonal cells (epithelial) surrounds the original fragment. No cells migrate out of the membrane into the medium. Culture B 114; Giemsa stain; magnification × 30.

FIG. 5. Border of the cell membrane of the same culture as seen in Fig. 4, highly magnified. The shape of the cells is shown in a firm medium; they are polygonal, even those situated at the edge of the membrane. Two of the cells are undergoing mitotic division. Culture B 114; Giemsa stain; magnification × 1,000.

PLATE 8.

FIG. 6. Frog skin, cultivated in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.); ten days after explantation. A dense membrane of polygonal epithelium-like cells surrounds the original fragment. The border of the membrane is uniform, and even the border cells are polygonal in shape. No cells are migrating from the membrane into the medium. Culture B 103; photographed while alive; magnification × 30.

FIG. 7. Frog skin, cultivated in a semi-firm medium (F.Pl. + F.E. + Ch.Pl.); about twenty hours after explantation. The cell membrane is dense only in the center; at the edge of the fragment it is loose, and here large pointed tongues of tissue project out into the medium. At the tips many spindle-shaped cells are in the act of becoming detached from the membrane and migrating into the plasma. Most of the cells situated at the edge have changed from being polygonal to fusiform. Therefore, the edges of the membrane are serrated, the medium being filled with free spindle-shaped cells. Culture B 86; Giemsa stain; magnification × 30.

PLATE 9.

FIG. 8. Frog skin, cultivated in a semi-firm medium (F.Pl. + F.E. + Ch.Pl.); three days after explantation. The same characteristics are seen as in Culture B 86 (Fig. 7), but more pronounced. There are many holes in the membrane at the back of the projections, produced by the forward motion of the cells into the medium, and they are not filled with fluid. Culture B 7; photographed while alive; magnification × 60.

FIG. 9. The polygonal cells at the edge of the cell membrane have assumed a spindle shape and become isolated from the membrane when cultivated in a soft medium. Drawing, made with a magnification, Leitz oc. 3; obj. 6; but without camera lucida.

FIG. 9 a. Culture A 406 c, cultivated in F.Pl. + F.E., six days after explantation. I. The homogeneous ectoplasm flows out into the medium. II. Cells a

and b assume a flask-like shape. III. Cell a has become isolated and contracted. IV. Cell a resumes the fusiform shape.

Fig. 9 b. Culture B 9, cultivated in F.Pl. + F.E. + Ch.Pl., four days after explantation. I. Cell a moves out into the medium, but remains connected by a thin plasma bridge with the other cells. II. Cell a assumes a round shape. III. Cell a resumes the spindle shape.

Plate 10.

Fig. 10. Frog skin, cultivated in a semi-firm medium (F.Pl. + F.E. + Ch.Pl.); twenty-one days after explantation. Only the most central zone of the membrane is compact, the borders are drawn out into long cloven tissue tongues, giving the culture a star shape. The migration of isolated spindle cells is considerable, and more extensive than in Figs. 7 and 8. Culture B 78; photographed while alive; magnification × 30.

Plate 11.

Fig. 11. The same culture as in Fig. 10, but twenty-two days after explantation. The cell membrane has further developed in size; there are long tissue projections and large holes in the cell membrane, some of which have been filled up with a softer medium. The hole in the upper part of the figure especially is bridged over by thread-like columnar cells. There are large numbers of isolated, migrating cells around the cell membrane, which spread out far into the medium. Culture B 78; Giemsa stain; magnification × 30.

Plate 12.

Fig. 12. Spindle-shaped cells of epithelial origin in a culture of frog skin, cultivated in a semi-firm medium (F.Pl. + F.E. + Ch.Pl.) for twenty-two days (same culture as in Fig. 11); the cells are migrating from the border of the tissue projections into the medium.

Fig. 12 a. Tip of a tongue of cells, projecting from the membrane into the medium, where the cytoplasm of the originally polygonal cells is seen flowing out into the medium, by means of which process the cells are assuming a spindle-like shape. The nuclei are also stretched out and elongated, and have a clear nuclear plasma and in addition one or two nucleoli, but no chromatin net, as is always the case in the polygonal cells, when fixed in formalin; the cytoplasm is more or less foamy in structure.

Plate 13.

Fig. 12 b. Two spindle cells shortly after isolation from the membrane, still connected with each other. Both possess some vacuoles in the cytoplasm, one of which is distinctly differentiated into a clear ectoplasm and a foamy endoplasm.

Fig. 12 c. Isolated and moving spindle cell. The anterior end, directed to the external edge of the drop of medium, is stretched out into a long, thin process, at the end of which a slight swelling has developed.

PLATE 14.

FIG. 13. Frog skin, cultivated in a soft medium (F.Pl. + F.E.); about twenty hours after explantation. Nearly the whole membrane is migrating away from the old fragment into the medium, in which we see little pieces of tissue moving freely. Holes of various sizes have developed in consequence of the migration of the border cells, the external parts of the membrane being connected with the internal ones by means of the thread-like columnar cells, which are long drawn out. The holes are not filled with a medium of different consistency from that prevailing outside, both being soft but not liquid. Nearly all the cells of the membrane, including the internal ones, are elongated; the cells at the edge are spindle-like. Culture B 119; photographed while alive; magnification × 30.

PLATE 15.

FIG. 14. Frog skin, cultivated in a soft medium (F.Pl. + F.E.); six days after explantation. Similar to Fig. 13. Isolated spindle-shaped cells and thread-like columnar cells can plainly be seen. Culture B 121; stained with Giemsa solution after fixation in formalin 2 per cent; magnification × 30.

PLATE 16.

FIG. 15. Frog skin, cultivated in a medium which is partly firm, and partly in the act of liquefying (F.Pl. + F.E.); four days after explantation. Large vacuoles are causing the cells to become stretched out, thus producing thread-like columnar cells. In the firm part of the medium a compact cell membrane is seen. The upper layers are separated from the fragment and form a thin membrane, which covers a part of the old fragment and part of the compact cell membrane. No change has been going on in these upper layers, the cells of which are neither growing nor moving. Culture B 117; photographed while alive; magnification × 30.

FIG. 16. Frog skin, cultivated in a soft medium (F.Pl. + F.E.); eighteen days after explantation. Only a very small, thin membrane remains around the old fragment. This membrane is extended into several tissue projections, from which the spindle-shaped cells are becoming separated and are migrating into the medium. The medium contains numbers of isolated cells, which are spindle-like or round. Culture B 77; photographed while alive; magnification × 30.

PLATE 17.

FIG. 17. Frog skin, cultivated in a soft medium (F.Pl. + F.E.), which had almost completely liquefied when the photograph was taken twenty-one days after explantation. The old fragment has become a globular ball. The cell membrane has entirely loosened up; in its place there are only isolated cells, most of which are round, but some are spindle-shaped. Culture B 81; photographed while alive; magnification × 30.

PLATE 18.

FIG. 18. A small fragment of a cell membrane, grown out of a piece of frog skin in a firm medium, isolated five days after explantation and kept for

two days in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.), at which time the picture was taken. A dense, compact membrane has grown out of the original fragment of membrane, consisting of polygonal cells. No migration of isolated cells into the medium is taking place. Culture B 100 a; photographed while alive; magnification × 30.

Fig. 19. A fragment of a cell membrane, grown out of a piece of frog skin in a soft medium, isolated four days after explantation, and kept in a soft medium (F.Pl. + F.E.) for about twenty hours, at which time the picture was taken. A network of elongated, spindle-shaped, and thread-like cells has developed from it, which greatly resembles connective tissue. The thread-like columnar cells are clearly seen in this photograph. Culture B 119 a; stained with Giemsa solution after fixation in 2 per cent formalin; magnification × 30.

Plate 19.

Fig. 20. Frog skin, cultivated in an unusually firm F.Pl. + F.E. medium. It possesses the same characteristics as a culture in a firm F.Pl. + F.E. + Ch.Pl. + Ch.E. medium (compare Figs. 1, 4, and 6). Culture A 157; about twenty hours after explantation; Giemsa stain, after fixation in 2 per cent formalin; magnification × 30.

Fig. 21. Frog skin, cultivated in an unusually firm F.Pl. + F.E. + Ch.Pl. medium; two days after explantation; the cell membrane and its cells, grown out of the old fragment, have the same characters as in a firm (F.Pl. + F.E. + Ch.Pl. + Ch.E.) medium (compare with Figs. 1, 4, and 6). Culture B 13; photographed while alive; magnification × 30.

Plate 20.

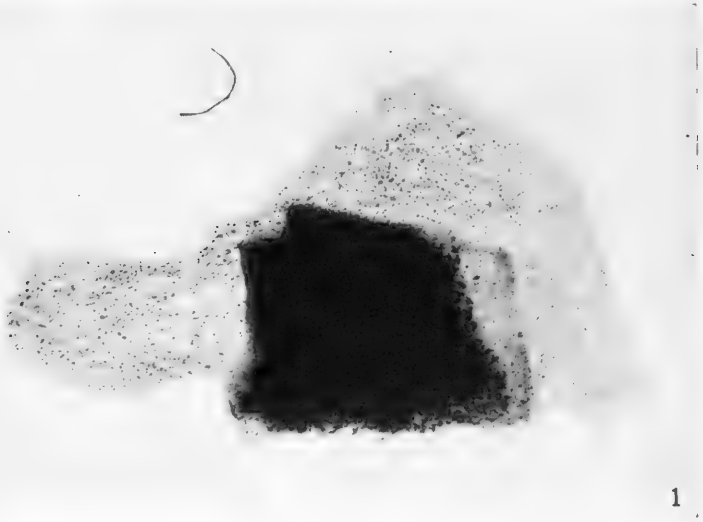
Fig. 22. Frog skin, which was cultivated in a firm medium (F.Pl. + F.E. + Ch.Pl. + Ch.E.); the membrane had for ten days the compact character peculiar to a firm medium. On the eleventh day the fresh medium, although of the same composition, had become softer and the membrane and the cells at once assumed the characteristics of cultures kept in semi-firm media of the composition of F.Pl. + F.E. + Ch.Pl., as is shown in this photograph (compare Figs. 7, 8, and 11). Culture B 100; eleven days after explantation; stained with Giemsa solution after fixation in 2 per cent formalin; magnification × 30.

Plate 21.

Fig. 23. Round cells. Fig. 23 a and b, from Culture B 81, which was placed in a liquid medium (compare Fig. 17), consisting of F.Pl. and F.E.; Fig. 23 c, from a semi-firm medium, consisting of F.Pl. + F.E. + Ch.Pl. + Ch.E.; fixed in formalin 2 per cent; Giemsa stain; camera lucida drawing; magnification, Leitz oc. 3; obj. 1/12, oil immersion; tube 170.

Fig. 24. Various stages of the movement of a formerly spindle-shaped cell, which is in process of becoming round; a twenty-two day old culture of frog skin, kept in a medium which is becoming liquid (F.Pl. + F.E. + Ch.Pl.). Culture B 45; drawing made from the living specimen; magnification, Leitz oc. 3; obj. 6; tube 160; schematic.

FIG. 25. Skin epithelium of a regenerating tail of the tadpole of *Rana pipiens,* showing a mitosis, the spindle of which is parallel with the surface of the connective tissue. Taken one day after the tip of the tail had been cut; fixed in potassium bichromate, 70 per cent; glacial acetic acid, 20 per cent; formalin, 10 per cent; stained with iron hematoxylin. Camera lucida drawing; half size; magnification, Leitz oc. 3; obj. 6; tube 160.



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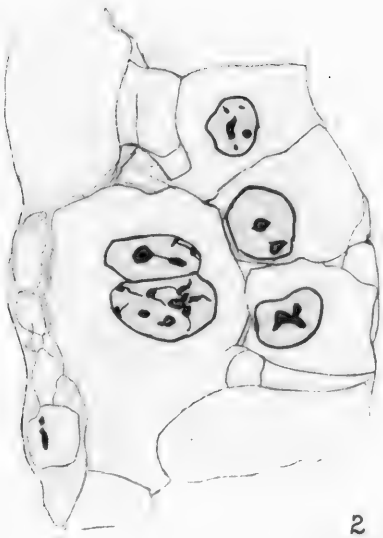

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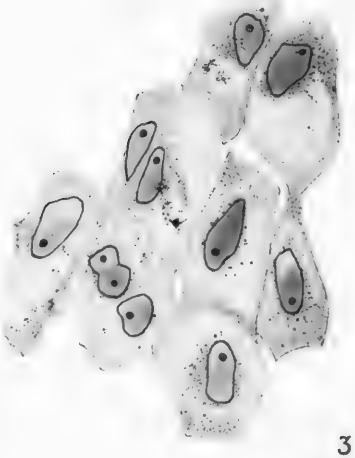

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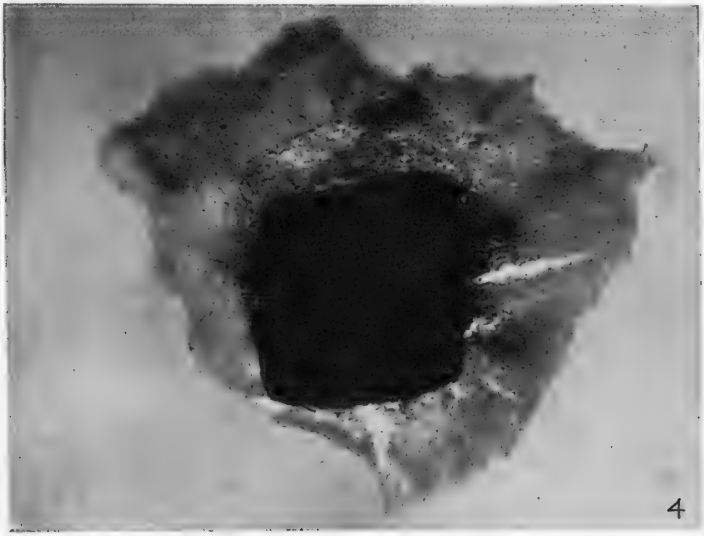

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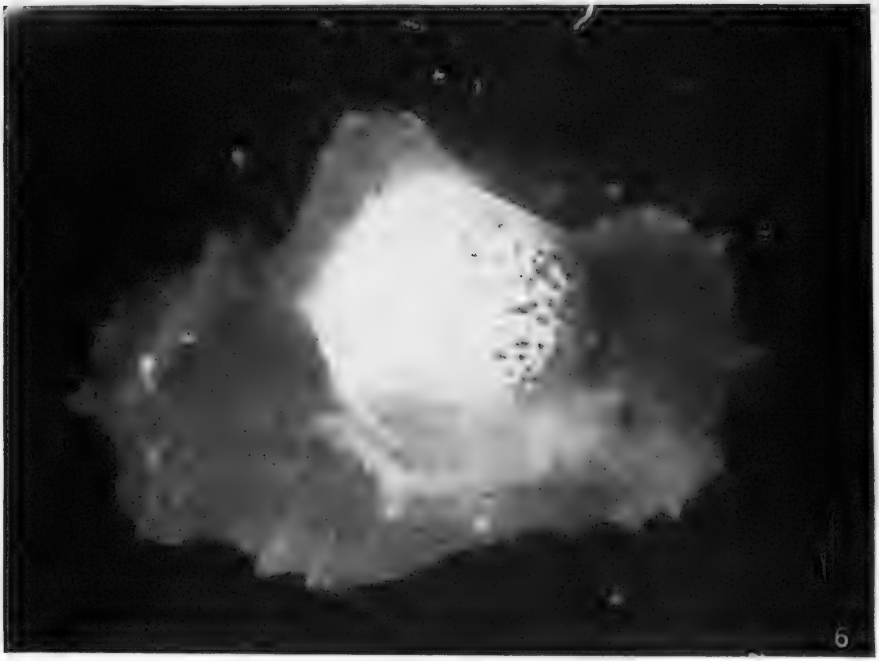

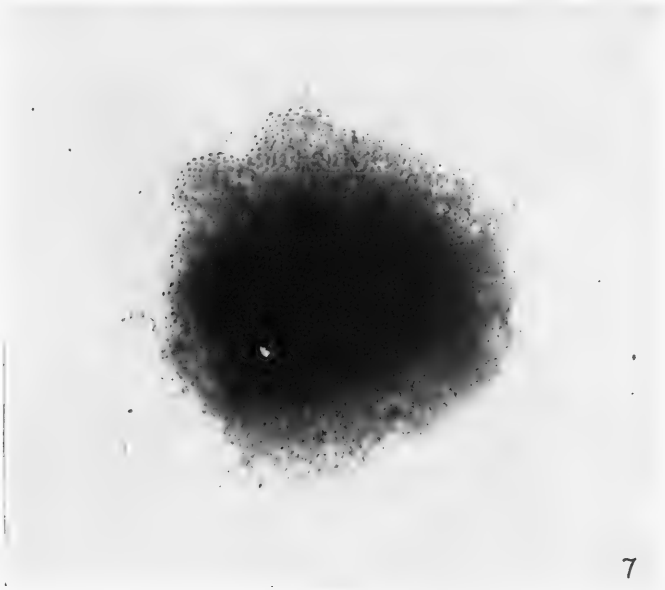

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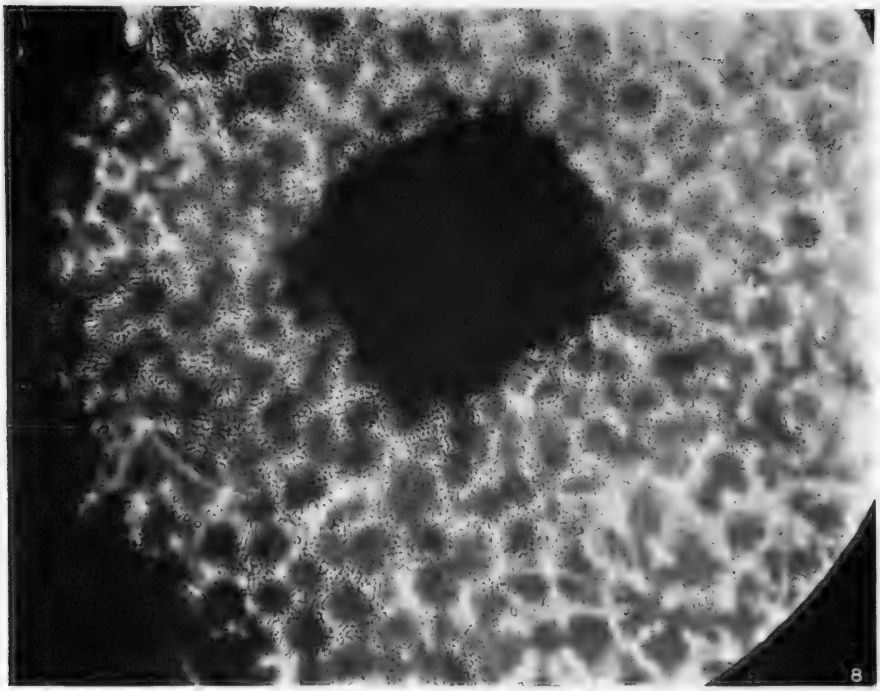

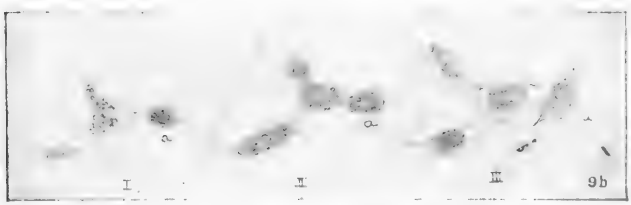

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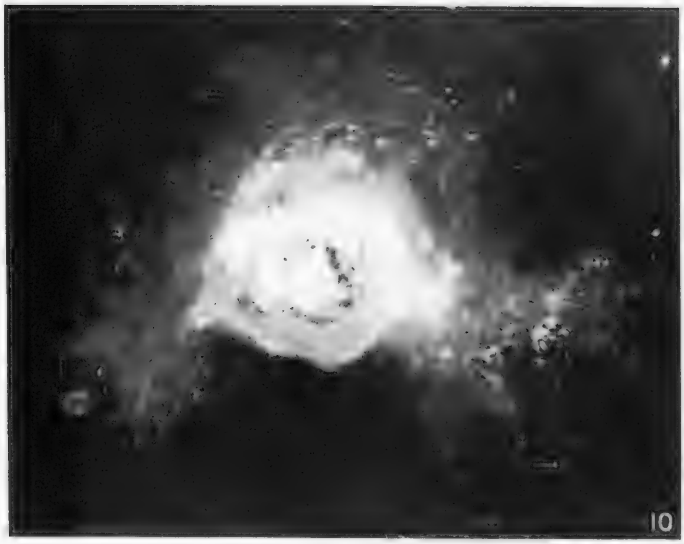

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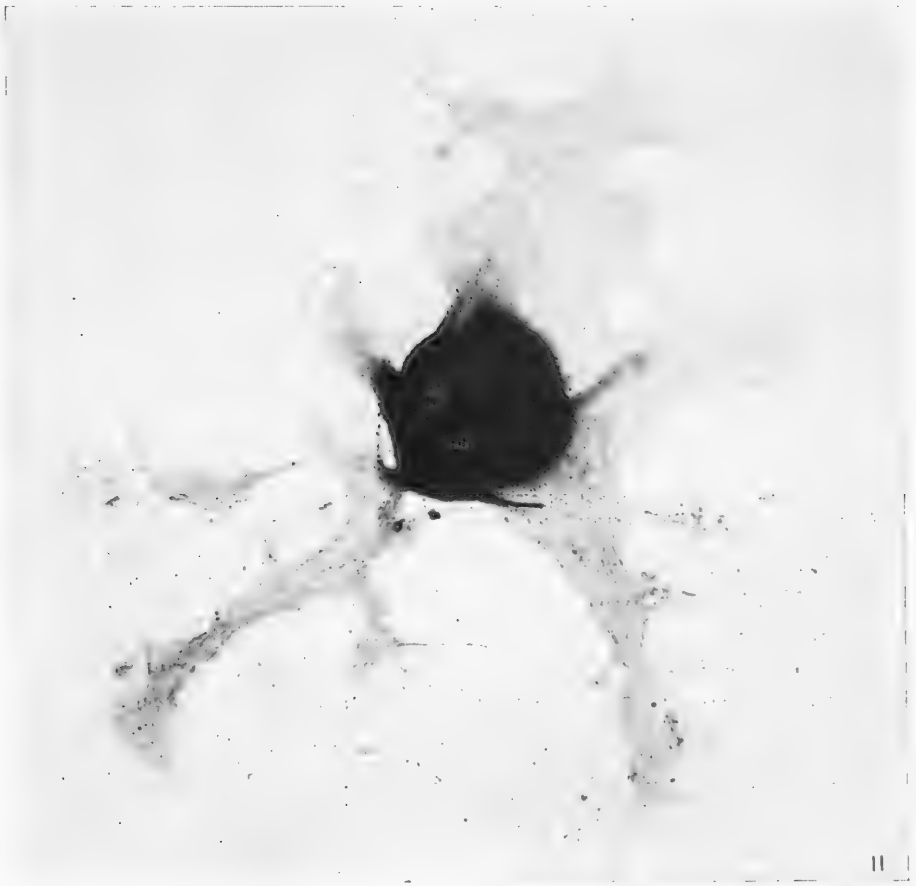

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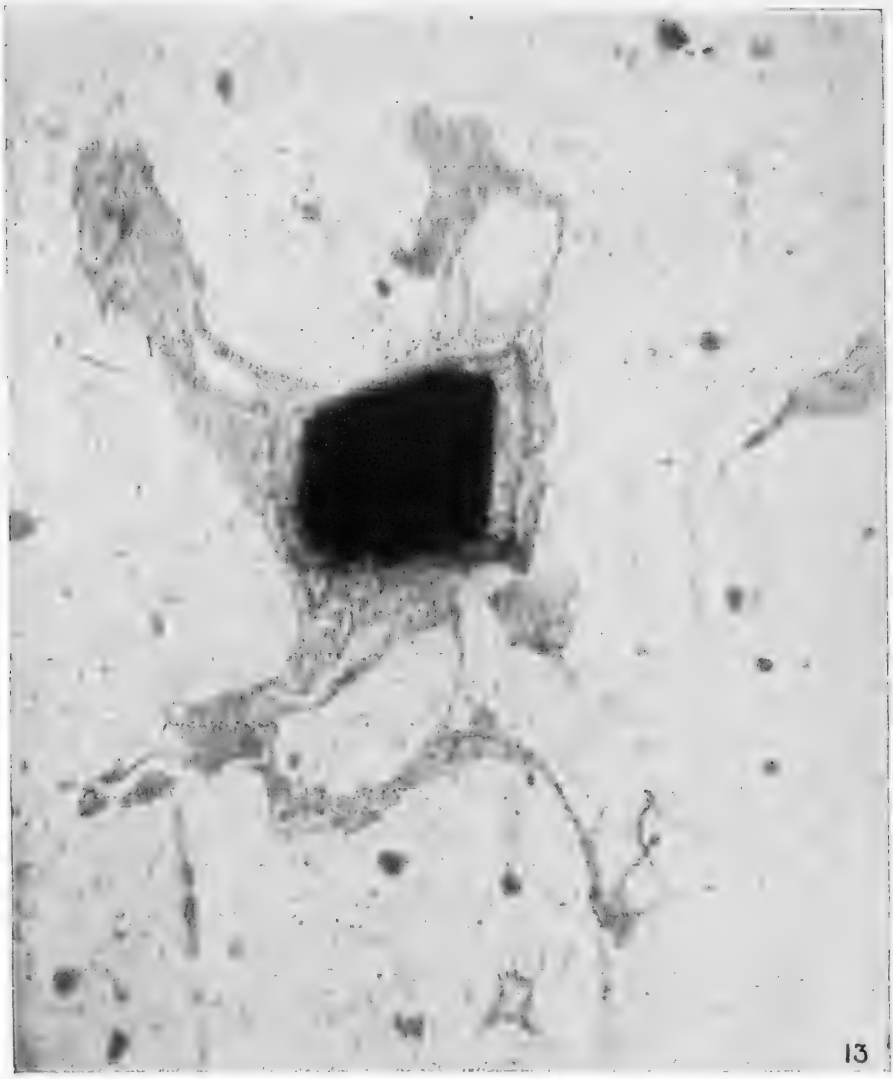

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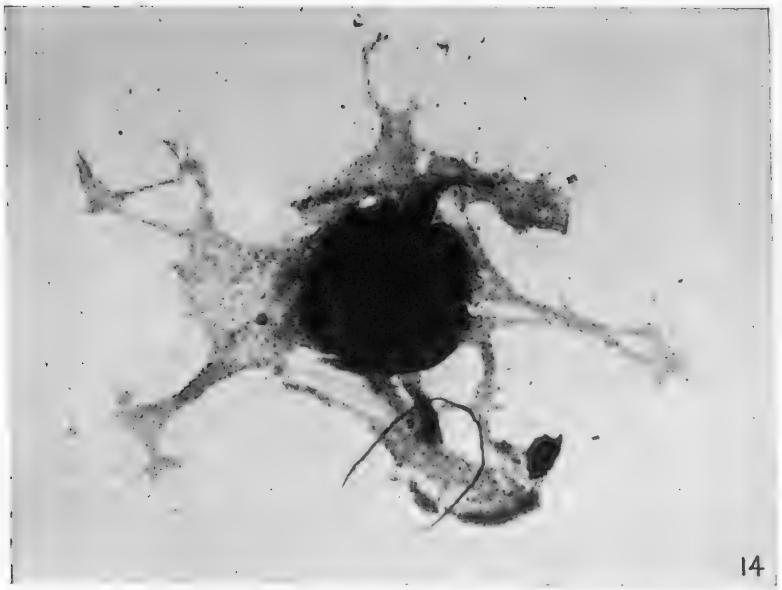

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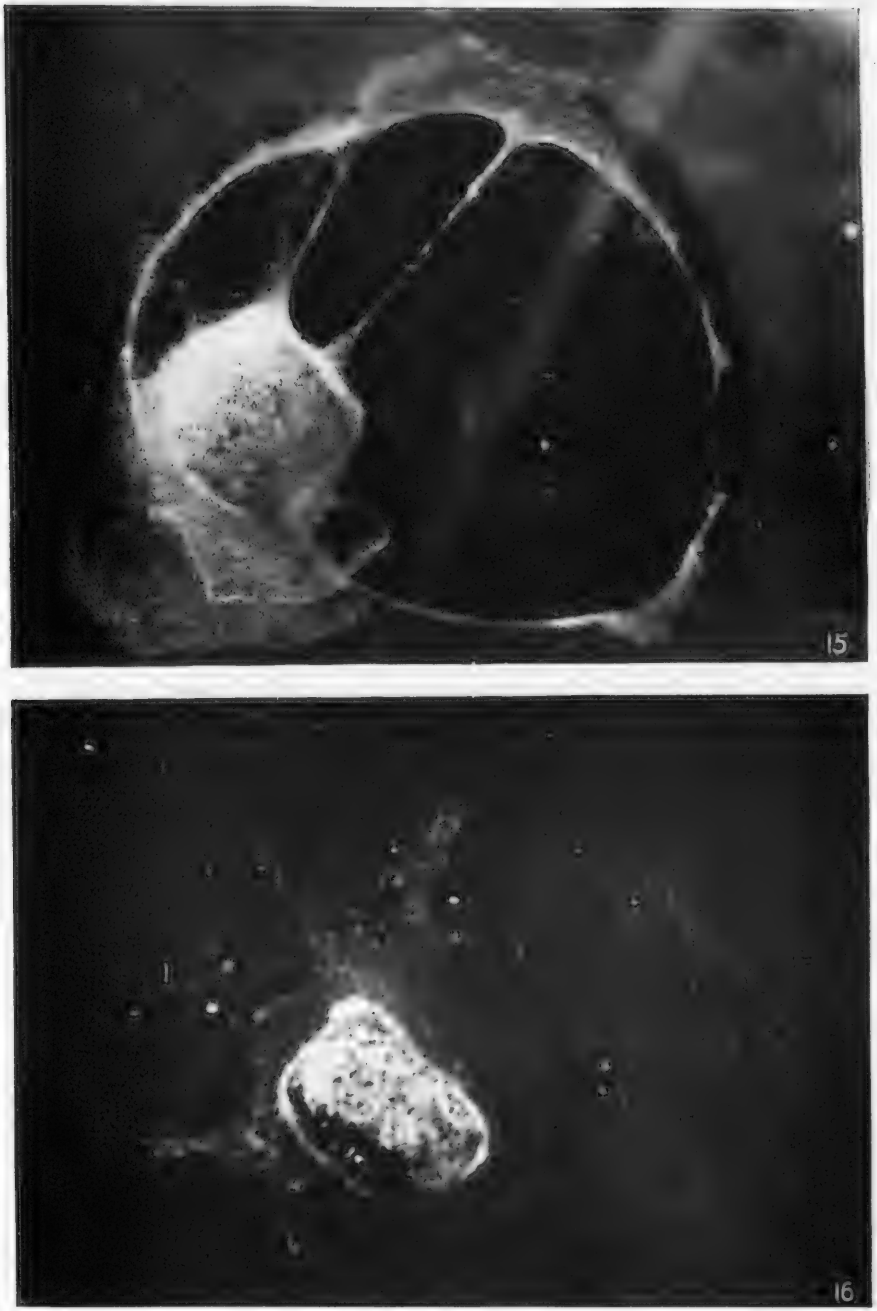

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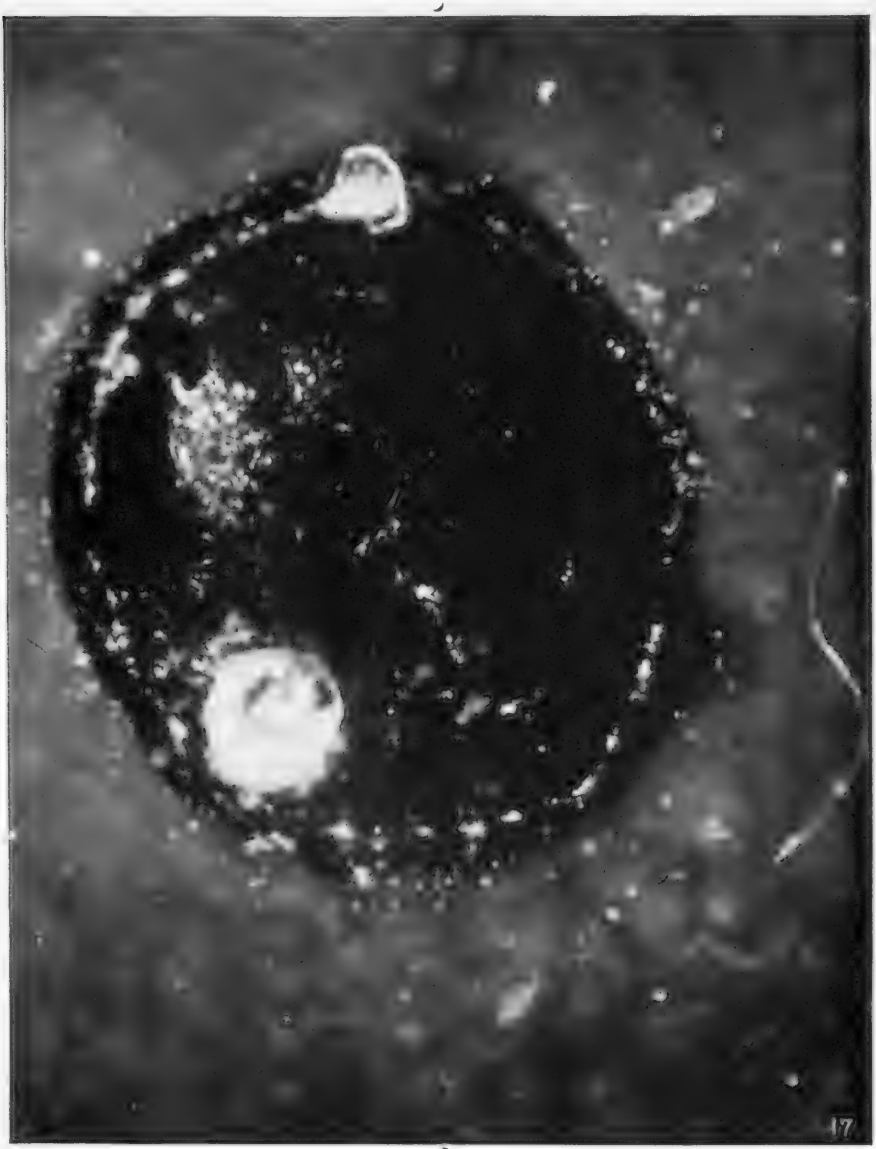

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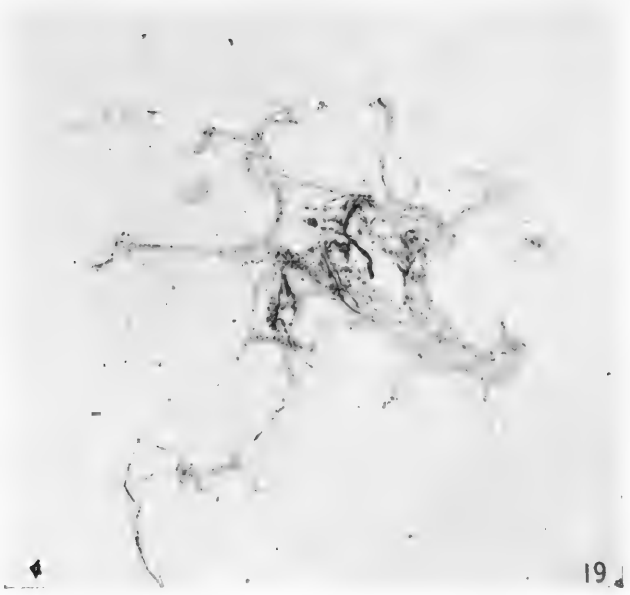

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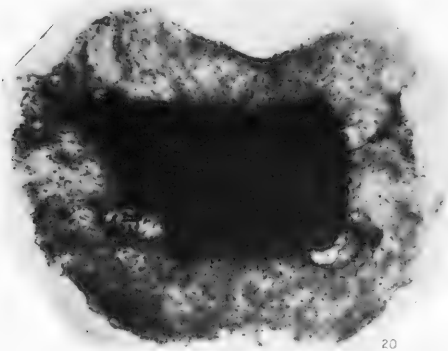

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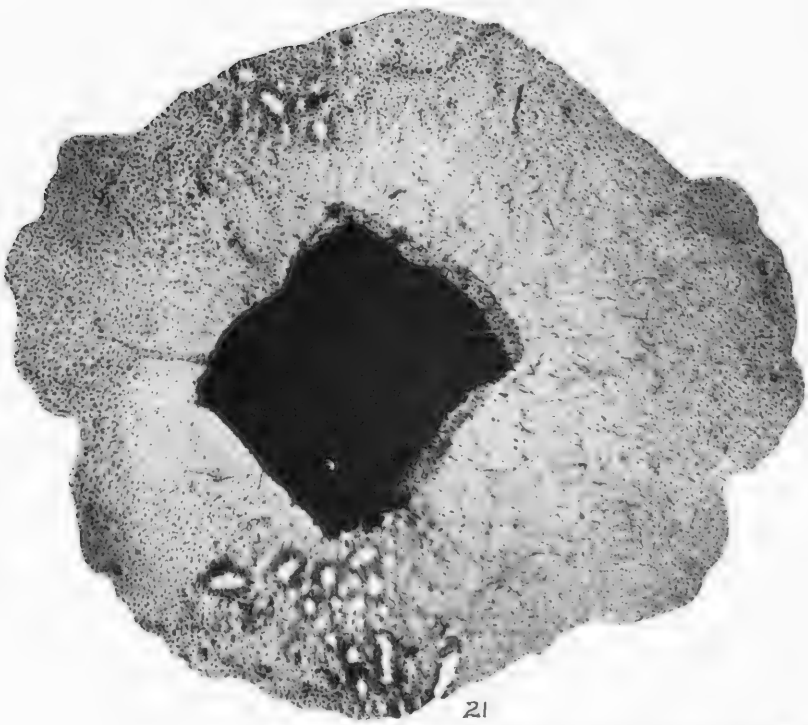

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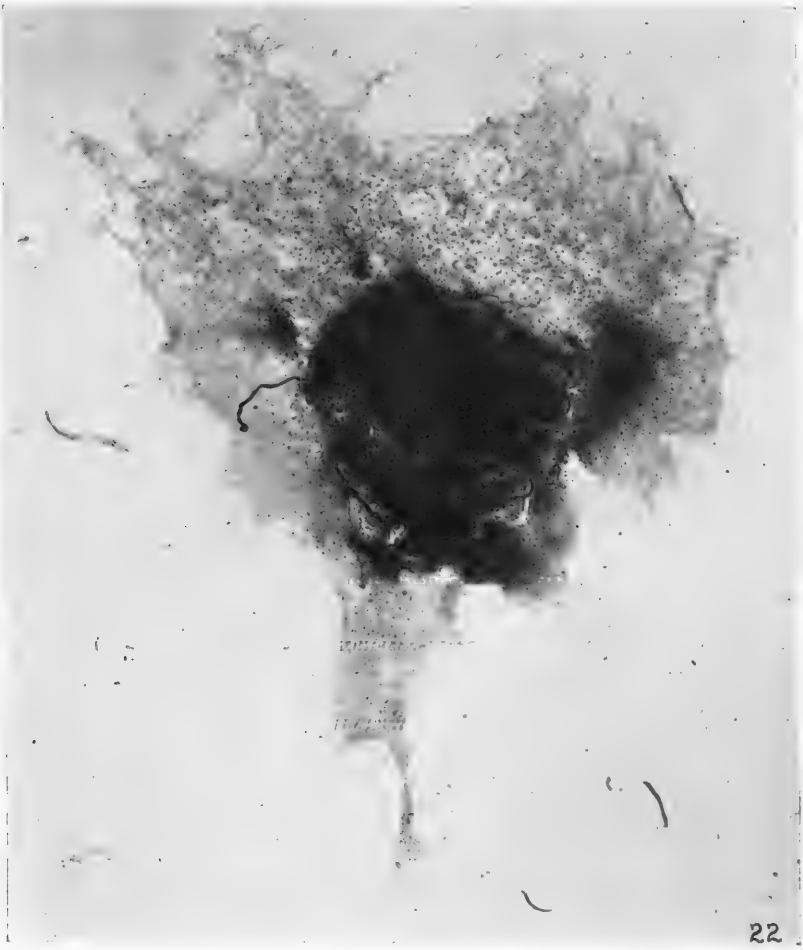

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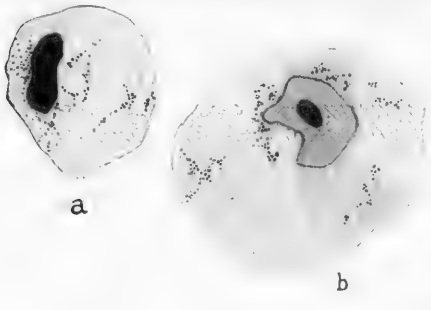

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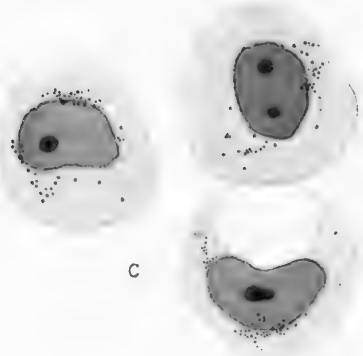

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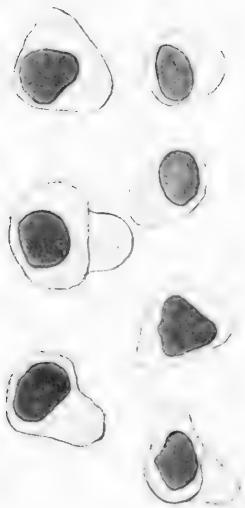

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THE OCCURRENCE OF CARRIERS OF DISEASE-PRODUCING TYPES OF PNEUMOCOCCUS.

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In previous papers[1] we have discussed the varieties of pneumococcus responsible for the production of lobar pneumonia and the differences that exist between such organisms and those found in the mouths of healthy individuals. The pneumococci obtained from persons suffering from lobar pneumonia have been divided into four groups. We have pointed out that the first three groups are comprised of disease-producing races, and are responsible for about 75 per cent of all cases of lobar pneumonia. Approximately 25 per cent of cases of pneumonia are due to the fourth group. The pneumococci of this group cannot readily be distinguished from those dwelling in the normal human mouth. Disease caused by the highly parasitic types is usually much more severe than that occasioned by the strains which are indistinguishable from the sputum pneumococci. Inasmuch as the highly virulent forms are always associated with disease, and only occur in the mouths of healthy individuals under the special conditions which are reported in this paper, the evidence is strong that in the spread of lobar pneumonia the disease in a majority of instances is transmitted from one individual to another. Infectious diseases usually spread by immediate contact, through the intermediation of a temporary host, or by the agency of a healthy carrier. The importance of these modes of transmission varies with different diseases and in many instances more than one of these mechanisms may be involved. In all likelihood the healthy carrier may be a greater menace to the health of the community than the

[1] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.*, 1913, lxi, 727. Dochez, A. R., and Avery, O. T., *Jour. Exper. Med.*, 1915, xxi, 114.

infected individual, largely because of failure to recognize the carrier condition. In the epidemiology of certain diseases, notably typhoid and epidemic cerebrospinal meningitis, the importance of the carrier state is well known because of the definite tracing of foci of disease to such a condition. The more readily a disease is transmitted from one individual to another, the greater becomes the number of instances at any one time, and consequently the more vigorous the search for the source of infection. The sporadic occurrence of lobar pneumonia, combined with the inability to distinguish disease-producing types of pneumococcus from those habitually living in normal mouths, has probably been responsible for the failure to establish a well defined epidemiology for this disease..

It is not our purpose in this paper to discuss the importance of immediate contact as a cause of the spread of pneumonia, for, although instances have occurred in which two closely associated individuals, such as husband and wife, have been infected within a short period with the same type of pneumococcus, cases of pneumonia usually develop at such wide intervals of space and time that direct contact relationships are obscured. Inasmuch as fully 75 per cent of all cases of lobar pneumonia are caused by peculiarly distinct races of pneumococci, not occurring in the normal mouth, then, as we have previously assumed, these instances of the disease must be due to contact infection, either direct or indirect, and some mechanism must exist by means of which the etiological agent is transmitted from one individual to another. In the present study we shall show that persons closely associated with individuals suffering from pneumonia in a large percentage of instances harbor in their mouths pneumococci of the same type as those causing the disease, that such organisms are not found in the mouths of normal individuals not exposed to pneumonia, and that the considerable period of time during which these organisms are carried may in part account for the sporadic occurrence of individual cases of pneumonia. An additional means of transmission exists in the fact that the recovered case also may carry the pneumococcus responsible for his disease during a relatively long period of time.

In the present paper are presented the studies of the pneumococci obtained from the mouths of persons associated with cases of pneumonia, of the type of pneumococcus encountered in the mouths of normal unexposed individuals, and of the period of time during which convalescents harbor the disease-producing organisms. For

TABLE I.

Incidence of Carrier Condition in Healthy Individuals in Contact with Lobar Pneumonia.

Case No.	Type of infecting pneumococcus.	Relationship of associates.	Type found in associates.	Duration of period of carrying.
2276	Type I	Wife	Type I	10 days+
2195	Type I	Husband	Type I	24 days.
2203	Type I	Wife	Type IV	
		Mother	Type IV	
2237	Type I	Nurse	Type I	Undetermined.
W	Type I	Wife	Type IV	
B	Type I	Wife	No pneumococcus	
		Daughter	Type IV	
2269	Type I	Wife	Type IV	
2286	Type II	Mother	Type IV	
		Father	Type IV	
2299	Type II	Sister	No pneumococcus	
2301	Type II	Wife	No pneumococcus	
2294	Type II	Wife	No pneumococcus	
2309	Type II	Mother	Type IV	
2314	Type II	Wife	Type II	Undetermined.
2335	Type II	Mother	No pneumococcus	
		Sister	No pneumococcus	
2326	Type II	Wife	No pneumococcus	
2339	Type II	Daughter	Type II	Undetermined.
2344	Type II	Sister	No pneumococcus	
1835	Type II	Wife	Type II	9 days+
2174	Type II	Son	Type IV	
2175	Type II	Mother	Type II	27 days+
		Sister	No pneumococcus	
		Nurse	Type II	Developed pneumonia, Type II.
2202	Type II	Mother	Type II	45 days.
2199	Type II	Husband	Type IV	
2208	Type II	Sister	Type IV	
2226	Type II	Wife	Type IV	
2245	Type II	Mother	Type IV	
		Brother	Type II	39 days+
2230	Type II	Wife	No pneumococcus	
2247	Type II	Daughter	Type II	Undetermined.
		Wife	No pneumococcus	
		Son	Type IV	
2266	Type II	Wife	No pneumococcus	
		Brother-in-law	No pneumococcus	
2270	Type II	Mother	Type II	7 days+
		Sister	No pneumococcus	
2265	Type II	Physician	Type II	21 days.

Summary.

TABLE I.—*Concluded.*

Type of pneumococcus.	No. of cases.	Positive contacts.	Per cent.
Type I.....	8	3	37.5
Type II.....	24	10	41.7
Total.....	32	13	40.6

Approximate duration of carrier state 23 days.

the sake of clearness, an explanation of our classification of pneumococci is given. The organisms have been placed into four groups, numbered from I to IV. Groups I, II, and III are found only in association with disease and are distinctly parasitic in type. Members of Groups I, II, and III are recognized by their immune reactions which are identical within the respective group. Group IV consists of a heterogeneous series of strains which are not related antigenically, and which cause a minority of cases of pneumonia, and from which the pneumococcus occurring in the normal mouth is indistinguishable. In the Tables I to IV the pneumococci studied are classified according to this numerical grouping.

TABLE II.

Type of Pneumococcus Isolated from Sputum of Normal Individuals.

Pneumococcus.		Incidence.	Per cent.
Contacts	Type I	3	2.6
	Type II	8	7.0
	Type IV	55	48.6
	No pneumococcus	47	41.6
	Total	113	
	Pneumococcus present	66	58.4
	Pneumococcus absent	47	41.6

TABLE III.

Type of Pneumococcus Isolated from Individuals with Lobar Pneumonia.

Pneumococcus.	No. of cases.	Per cent.
Type I	78	34.97
Type II	75	33.63
Type III	22	9.86
Type IV	48	21.52
Total No. of cases	223	

TABLE IV.

Persistence of Disease-Producing Type of Pneumococcus during Convalescence.

Case No.	Type of pneumococcus during height of disease.	Type of pneumococcus after recovery.
1654	Type I	60 days, Type I.
		65 days, Type IV.
1751	Type I	59 days, streptococcus.
		73 days, streptococcus.
1775	Type I	30 days, Type IV.
1867	Type I	90 days, Type I.
1828	Type I	33 days, Type IV.
1792	Type I	30 days, Type IV.
2167	Type I	12 days, Type IV.
2168	Type I	45 days, Type I.
2191	Type I	15 days, Type IV.
2195	Type I	28 days, Type I.
		49 days, no pneumococcus.
2203	Type I	12 days, no pneumococcus.
2237	Type I	13 days, Type I.
W	Type I	15 days, Type I.
		23 days, Type IV.
B	Type I	14 days, no pneumococcus.
2250	Type I	10 days, Type I.
2269	Type I	13 days, no pneumococcus.
2284	Type I	15 days, Type I.
		29 days, no pneumococcus.
2267	Type I	15 days, no pneumococcus.
2276	Type I	15 days, Type I.
1679	Type II	30 days, streptococcus.
		48 days, Type IV.
		108 days, Type IV.
1753	Type II	60 days, Type IV.
1763	Type II	34 days, streptococcus.
		40 days, Type IV.
1761	Type II	47 days, streptococcus.
		78 days, Type IV.
1825	Type II	53 days, Type II.
1786	Type II	20 days, Type IV.
		25 days, Type IV.
1820	Type II	30 days, Type II.
1827	Type II	14 days, Type IV.
1880	Type II	63 days, Type II.
1950	Type II	21 days, Type IV.
1969	Type II	24 days, Type IV.
2174	Type II	15 days, streptococcus.
2175	Type II	17 days, no pneumococcus.
2212	Type II	12 days, Type II.
		17 days, no pneumococcus.

TABLE IV.—*Concluded.*

Case No.	Type of pnemococcus during height of disease.	Type of pneumococcus after recovery.
2202	Type II	37 days, Type II.
		43 days, Type IV.
2199	Type II	32 days, no pneumococcus.
2226	Type II	29 days, Type II.
2245	Type II	16 days, Type IV.
2266	Type II	15 days, Type II.
2270	Type II	37 days, Type II.
2286	Type II	10 days, Type II.
		25 days, no pneumococcus.
2292	Type II	29 days, Type IV.
2296	Type II	19 days, no pneumococcus.
1743	Type III	13 days, Type III.
		73 days, Type IV.
2185	Type III	16 days, Type III.
		34 days, no pneumococcus.
2249	Type III	14 days, Type III.

In the above four tables are given the main facts upon which we base our assumption that in the majority of cases lobar pneumonia is a disease the continued wide-spread incidence of which is dependent upon communication of infection from one individual to another. Table I establishes beyond doubt the existence of healthy carriers of the disease-producing types of pneumococcus. The study of the carrier state was limited to the investigation of infection with pneumococcus Types I and II because of the relative ease with which these organisms can be distinguished from other types of pneumococcus. Out of a total of thirty-two cases studied, at least one carrier of infection among the patient's associates was found in thirteen instances, 40.6 per cent. Types I and II show approximately the same percentage incidence of the carrier condition, and in every instance the pneumococcus isolated corresponds in type with that of the infected individual. The approximate duration of the carrier state has been twenty-three days, which is probably somewhat shorter than would be found had it been possible to retain under observation all carriers until the disease-producing type of pneumococcus had disappeared from the mouth

flora. Study of Table I shows that positive carriers are more commonly observed among females than among males, a fact that is probably accounted for by the more frequent service of the former in a nursing capacity, thus entailing more intimate association with the sick.

Tables II, III, and IV develop somewhat further, points brought out in a previous communication. In Table II is shown the incidence of pneumococcus in the mouth flora of normal individuals and the classification of such pneumococci. All normal individuals studied are given in this table, whether in contact with cases of lobar pneumonia or not. Pneumococcus was found in 58.4 per cent of all instances and was absent in 41.6 per cent. Of the cases in which pneumococcus has been found, 48.6 per cent of the organisms have been of the sputum type and 9.6 per cent have been of the disease-producing type. All the latter have occurred in individuals intimately associated with cases of lobar pneumonia, and their presence is dependent upon this association. Determination of contact carriers of pneumococci belonging to Group III, *Pneumococcus mucosus,* presents certain difficulties. Only recently have we been able to obtain an immune serum effective against organisms of this group. Further development of the study of the mucous group is showing that certain organisms resembling in their cultural reactions *Pneumococcus mucosus,* are, in reality, mucous types of streptococcus. The latter varieties are frequently found in normal sputum, and have led to some confusion. With the working out of the serological reactions of the true *Pneumococcus mucosus,* evidence is accumulating that this organism is as strictly pathogenic in type, and has quite as specific immunological characteristics as pneumococci belonging to Groups I and II.

For comparison with the statistics given in Table II, which illustrates the prevalence of the mouth type of pneumococcus in normal individuals, Table III is added to show the percentage incidence of the fixed types of pneumococci observed in individuals suffering from lobar pneumonia. In this table the highly pathogenic types are dominant, being responsible for 77.2 per cent of all infections studied. This fact is convincing evidence that specific types of

pneumococci are mainly responsible for the production of lobar pneumonia. The dominance of these organisms in disease is in striking contrast to the high percentage incidence of the sputum type of pneumococcus in the mouth flora of normal individuals.

Table IV shows the length of time during which recovered cases of pneumonia harbor the organism responsible for their disease. The period of carrying is measured from the date of onset of the pneumonia. The shortest time in which the disease-producing pneumococcus has disappeared from the mouth has been twelve days, and the longest duration of carrying has been ninety days. These results show that pathogenic types of pneumococcus persist in the mouths of individuals recovering from lobar pneumonia for a variable period of time. Because of wide intervals between observations, the average duration of this condition can only be approximately determined, and has been found to be about twenty-eight days.

The studies detailed in this paper show that there are two sources of danger in the spread of pneumococcus infection. One lies in the occurrence of healthy carriers of disease-producing pneumococci among individuals associated with cases of pneumonia, and the other in the fact that patients recovering from the disease harbor the responsible organism for a considerable length of time. The actual tracing of cases of pneumonia to examples of the carrier state is difficult, but even the small amount of effort that has so far been devoted to this side of the study has brought to light an occasional suggestive fact.

SUMMARY.

Lobar pneumonia in 75 per cent of instances is due to specific types of pneumococci possessed of a high degree of pathogenicity. Although pneumococci occur in the mouths of 60 per cent of normal individuals, such organisms are readily distinguishable from the highly parasitic types of pneumococcus responsible for the severe forms of lobar pneumonia, a convincing proof that infection in this disease is, in the majority of instances, not autogenic in nature, but is derived from some extraneous source. In a high percentage of instances healthy persons intimately associated with

cases of lobar pneumonia harbor the disease-producing types of pneumococcus. In every such instance the pneumococcus isolated has corresponded in type with that of the infected individual. Convalescents from pneumonia carry for a considerable length of time the type of pneumococcus with which they have been infected. The existence of the carrier state among healthy persons and among those recently recovered from pneumonia establishes a basis for understanding the mechanism by means of which lobar pneumonia spreads and maintains its high incidence from year to year.

FURTHER EXPERIMENTS ON THE EFFECTS OF LONG CONTINUED INTRAPERITONEAL INJECTIONS OF PROTEINS.

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In a previous article[1] we said: "Albumose (Witte's peptone) introduced parenterally into the guinea pig has very little, if any, harmful effect unless the oxidative powers of the organism are below normal. In view of the results of Longcope's experiments, as compared with ours, it seems possible that the more complex proteins will produce effects in the absence of decreased oxidation which the less complex ones will not produce under similar circumstances." These remarks were based upon a short series of experiments in which seven guinea pigs were given intraperitoneal injections of 1 per cent solution of Witte's peptone. Since the publication of this series we have continued the work, as will be detailed hereafter.

The different experiments are grouped as follows: 1. Guinea pigs which were given only Witte's peptone (Nos. 1, 2, 3, 4, 5, 18, 19). 2. Guinea pigs which received Witte's peptone, followed by chloroform anesthesia for fifteen minutes (Nos. 11, 12, 21, 22). 3. Guinea pigs which received chloroform only (Nos. 24, 27, 29). 4. Controls (Nos. 20, 23). 5. White rats which received intraperitoneal injections of a solution of albumen (Nos. 12, 13). 6. White rats which received intraperitoneal injections of a solution of albumen together with subcutaneous injections of chloroform in oil (Nos. 21, 22). 7. White rats which received intraperitoneal injections of casein (Nos. 14, 17). 8. White rat which received intraperitoneal injections of casein together with subcutaneous injec-

[1] Woolley, P. G., DeMar, A., and Clark, D., *Science*, 1914, N.S., xl, 789.

tions of chloroform in oil (No. 16). 9. Controls (Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10).

In the protocols the expression albumose solution means one prepared according to the following formula and then sterilized:

Witte's peptone	1.0 gm.
Sodium chloride	0.5 gm.
Distilled water	100.0 gm.

The albumen solutions were made by dissolving 10 mg. of desiccated egg-albumen in 2 cc. of distilled water. The solutions were freshly prepared before each inoculation.

The chloroform solution was a 2 per cent solution in sterile olive oil.

The casein was given in the form of a solution made by dissolving 1 gm. of casein in 100 cc. of normal sodium hydrate and then carefully neutralizing (to phenolphthalein) with normal hydrochloric acid.

Group I.

Guinea Pig 1.—Weight 400 gm. This animal received 17 daily injections each of 1.5 cc. of the peptone solution, a total of 25.5 cc., or 0.255 gm. of albumose. It died suddenly on the day following the last injection. The cause of death was not discovered. The postmortem examination was done while the animal was still warm, and showed no other changes than a slight mediastinal edema, moderate hyperplasia of the lymph nodes, and congestion of the lungs, liver, spleen, and kidneys. Microscopic examination of the tissues showed edema and congestion with occasional small hemorrhages in the kidneys, with a few areas of small round cell infiltration, enormous congestion of the adrenals, edema and focal necroses of the thymus, and hyperplastic changes associated with congestion in the lymph glands and spleen. The spleen was more than normally pigmented.

Guinea Pig 2.—Weight about 350 gm. This animal received 57 daily injections each of 1.5 cc. of the peptone solution, a total of 85.5 cc., or 0.855 gm. of albumose. During the period of treatment it gave no sign of any untoward effects of the treatment. It ate well, lost no weight, and was finally chloroformed 72 hours after the last injection. The postmortem examination was done while the body was still warm. The organs showed no abnormal macroscopic or microscopic lesions, other than a moderate, generalized congestion associated with a very moderate edema of the parenchymatous organs. This, however, was no more than is usual after chloroform anesthesia.

Guinea Pig 3.—Weight about 400 gm. This animal received 30 daily intraperitoneal injections each of 1.5 cc. of the peptone solution, a total of 5 cc., or 0.45 gm. of albumose. It was killed with chloroform. The postmortem exami-

nation showed only a very moderate congestion and edema of the liver, spleen, kidneys, and adrenals, and a slight hyperplasia of the mesenteric lymph glands. Microscopic examination showed nothing abnormal except perhaps a slight degree of hyperplasia of the mesenteric lymph glands.

Guinea Pig 4.—Weight about 400 gm. This animal received 5 cc. of the peptone solution each day for 7 days, a total of 35 cc., or 0.35 gm. of albumose. It was killed with chloroform. At autopsy nothing abnormal was found. Microscopic examination was also negative.

Guinea Pig 5.—Weight about 350 gm. This animal was treated in the same manner as No. 4 for a period of 20 days, during which time it received a total of 100 cc. of the peptone solution, or 1 gm. of albumose. It was chloroformed and autopsied. During the period of treatment it lost 87 gm. in weight. At autopsy nothing noticeable was found except a partially healed meager exudate on the surface of the spleen. The peritoneal cavity contained 2 cc. of a clear fluid. Microscopic examination of the tissues showed no lesions except in the case of the spleen, in which there was an increased amount of pigment and a moderate hypertrophy of the Malpighian follicles. The capsule was thickened.

Guinea Pig 18.—Weight 470 gm. This animal received 5 cc. of the peptone solution, intraperitoneally, each day for 42 days, a total of 210 cc., or 2.1 gm. of albumose. At the time of death it weighed 485 gm., a gain of 15 gm. It was killed with chloroform. The organs showed but little change. The spleen was congested; the liver and kidneys showed occasional foci of small round cells. The peritoneal cavity contained no free fluid.

Guinea Pig 19.—Weight 452 gm. This animal received exactly the same treatment as Guinea Pig 18. At the end of the experiment the weight had dropped to 420 gm., a loss of 32 gm. The organs showed more noticeable microscopic changes than those of Guinea Pig 18. In the kidney the small round cell infiltration was rather diffuse,—the foci of accumulation were more numerous. The liver was unusually congested. The spleen was diffusely hyperplastic. The adrenal exhibited small round cell infiltration in foci in the medulla, and the cortex showed an unusual amount of lipoid metamorphosis. In the medulla there was an increase of hyaline material.

Remarks.—This group of experiments seems to show that comparatively large doses of albumose introduced in small doses over a considerable period of time have practically no harmful effect upon the organs of the body, unless it may be that the foci of small round cells are due to the treatment. It is possible that were the experiments continued over very long periods of time these foci might become fibroid.

Group II.

Guinea Pig 12.—Weight 412 gm. This animal received 50 intraperitoneal injections of 5 cc. of the peptone solution in the course of two months, a total of 250 cc., or 2.5 gm. of albumose. After each injection it was submitted to

deep chloroform anesthesia for 15 minutes. After 25 treatments the weight had increased to 455 gm. At the end of the treatments the weight was 485 gm. The postmortem examination revealed nothing macroscopically abnormal, and physically the animal seemed to be in good condition. Histological examination showed that there was a certain amount of anatomic modification of the tissues of some of the organs. The report was as follows: The kidney shows a well marked edema and cloudy swelling. The glomerular spaces are dilated and the tufts compressed, and in the spaces there is considerable coagulated albuminous material. About the glomeruli there are frequent small accumulations of small round cells, and in the outer layers of the cortex there are occasional lines of interstitial fibrosis. The whole organ showed congestion. The liver showed a very well developed edema, to the extent that in many areas the cells show what seem to be hydropic changes. With this is associated congestion and very moderate interstitial fibrosis as exemplified in the occasional collections of small round cells in the perilobular connective tissues. The spleen shows enormous hyperplasia of the Malpighian follicles together with some increased pigmentation. Within the corpuscles there is evidence of cellular fragmentation. The adrenals show a few collections of formative cells in both medulla and cortex, chiefly in the latter. The other organs revealed nothing remarkable.

Guinea Pig 11.—Weight 445 gm. This animal was treated in exactly the same way as No. 12. After 25 treatments it weighed 482 gm., and at the end of the experiment 565 gm. The report of the histologic examination stated that the changes were similar to those found in No. 12, except that there were a few retention cysts in the kidneys and that there was nothing of note in the adrenals except an intense congestion.[2]

Guinea Pig 21.—Weight 365 gm. This animal received 40 daily injections (intraperitoneal) each of 5 cc. of the albumose solution, and each injection was followed by 15 minutes of deep chloroform anesthesia. At the end of the experiment the weight had fallen to 340 gm., a loss of 25 gm. It was killed with chloroform. The cellular and organic changes were practically the same as those exhibited by Guinea Pig 19, though somewhat less in degree.

Guinea Pig 22.—Weight 340 gm. This animal was treated in exactly the same manner as Guinea Pig 21. At the end of the experiment it weighed 360 gm., a gain of 20 gm. The sections made from the organs showed changes similar to those described in Guinea Pig 18.

Remarks.—It was the results obtained in the first two animals in this group which, compared with those of Group I, called forth the remarks quoted in the first paragraph of this report. Chloroform undoubtedly reduces the oxidations of the body, and when administered at the same time with other substances may lead to the production of grave changes in the tissues, as Opie[3] has shown. It

[2] The histologic examinations in these cases were made by Dr. T. H. Kelly, who had no knowledge of the experimental procedures used in the individual cases. They were subsequently verified by one of us.

[3] Opie, E. L., *Tr. Assn. Am. Phys.*, 1910, xxv, 140.

is, however, to be remarked at this place that the results in the last two animals do not support the conclusion that because chloroform may do these things, it must do them. What conditions were present in Animals 11 and 12 to cause them to react differently, we cannot tell. They were from a different lot of animals from Nos. 21 and 22; they were fed more liberally with fresh vegetables than the latter, because they were procured in the late summer. But, on the other hand, they were of the same lot as Nos. 1, 2, 3, 4, and 5. Nos. 21 and 22 belonged in the same lot as 18 and 19. Still, perhaps the effect is dietary.

Group III.

Guinea Pig 24.—Weight 340 gm. This animal was treated on successive days by deep anesthetization for 15 minutes. At the end of the experiment it had lost 55 gm. It was killed by complete anesthetization. The tissues showed no changes other than congestion.

Guinea Pig 27.—Weight 616 gm. This animal was treated in exactly the same manner as was Guinea Pig 24, except that the number of anesthetizations was 9. After the 9th treatment it weighed 620 gm. The organs were similar to those of Guinea Pig 24.

Guinea Pig 29.—Weight 358 gm. This animal was treated like the two preceding, except that it received 40 treatments. At the end of the experiment it weighed 435 gm., a gain of 77 gm. The organs showed no macroscopic abnormality. Sections showed intense fatty metamorphosis of the liver and little else.

Remarks.—Apparently chloroform alone produces no more change, so far as interstitial changes are concerned, than the combination of chloroform and albumose. It does reduce oxidations and therefore tends to bring about an increase in weight, largely perhaps because of a decrease in oxidation of the fats.

Group IV.

Guinea Pigs 20 and 23.—These were normal controls, the organs of which were used for comparison. In each there were occasional fibrotic glomeruli in the kidney and occasional foci of small round cell infiltration.

Remarks.—The preceding group of experiments brings out no revolutionary fact. They merely indicate that while albumose administered, as we have done it, to normal guinea pigs produces, as a rule, no obvious symptoms or histologic changes, even when chloro-

form is given at the same time, yet occasionally early sclerotic changes appear.

Group V.

White Rat 12.—Weight 286 gm. This animal received 25 injections on successive days, each of 5 mg. of albumen. At the end of the experiment it weighed 305 gm. Postmortem examination showed no gross abnormality. Sections showed excessive edema and cloudy swelling, and in some of the glomerular spaces small mounts of coagulated albumen.

White Rat 13.—Weight 225 gm. This animal was treated in the same manner as Rat 12, except that it was given 44 injections, a total of 220 mg. of albumen. At the end of the experiment the weight had fallen 40 gm. The animal was killed by a blow on the neck.

The organs were apparently normal, macroscopically. The sections of the organs showed a considerable degree of cloudy swelling, especially of the convoluted tubules. In places the appearances were almost those of necrosis. Otherwise there were no microscopic changes, except a small granulomatous area in the liver.

Remarks.—The only essential difference in the tissues of Rats 12 and 13 lay in the presence of albumen in the glomerular spaces in No. 12, which was killed with chloroform. In No. 13 the marked cloudy swelling was perhaps due to whatever produced the granuloma.

Group VI.

White Rat 21.—Weight 282 gm. This animal received 40 daily injections of 5 mg. of albumen, intraperitoneally, and subcutaneous doses of 0.5 cc. of a 2 per cent chloroform solution in oil. At the end of the series it had lost 12 gm. in weight. Neither the organs nor the sections showed anything remarkable.

White Rat 22.—Weight 273 gm. This animal was treated for 7 days, like Rat 21. It lost no weight. The organs showed merely edema.

Group VII.

White Rat 14.—Weight 163 gm. This animal received 25 injections, on successive days, of 10 mg. of casein. At the end of the experiment it weighed 190 gm. Nothing anatomic or histologic seemed to result from the treatment.

White Rat 17.—Weight 290 gm. This animal received daily intraperitoneal injections of 10 mg. of casein for 17 days, followed by 23 similar injections each of 70 mg. of casein. At the end of the series it had lost 20 gm. There were no essential changes observed, macroscopically or microscopically, in the organs or tissues.

Group VIII.

White Rat 16.—Weight 310 gm. This animal was treated exactly like Rat 17, except during the last 23 days, when it was receiving 20 mg. of casein daily.

It also was given 0.5 cc. of the chloroform oil solution daily. At postmortem examination there was no evidence of gross changes, and sections showed nothing more than marked congestion and cloudy swelling.

Group IX.

White Rats 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.—These animals of various weights and ages furnished material for comparison.

Rat 1 had been fed on a strictly meat diet for several weeks.

Rat 2 had been fed on grain and vegetables for several weeks.

Rats 4 and 5 had been injected with trypan blue.

Rats 6, 7, 8, 9, and 10 had been given a single injection of uranium acetate, and had been killed after 24 to 48 hours.

DISCUSSION.

As stated in our earlier report, it was our object in carrying out this series of experiments to determine, if possible, the organic effects of certain substances of protein nature which might be produced within the tissues, or which might be absorbed from the intestinal tract.

Longcope[4] has said that parenteral digestion of egg-albumen may (under certain circumstances) seemingly produce organic renal and hepatic changes. This may be taken to mean that splitting of the whole protein (egg-albumen) leads to the production of these phenomena; that the effects are the results of the irritant action of substances set free during splitting; or that moieties of the protein molecule, by making abnormal combinations, may act as irritants or in some other way embarrass the functional cells of the tissues and at the same time stimulate the overgrowth of fibrous tissue.

Longcope produced the effects which he reported, not by the mere administration of albumen, but by so timing the injections that a mild state of anaphylaxis resulted. In our series we have not done this, but have endeavored to discover whether the substances which we have used would produce similar effects in the absence of anaphylaxis, and whether, at the same time, the addition of materials (such as chloroform) which hinder the oxidations in the cells, would modify or intensify the reactions. In a former series of experiments Woolley and Newburgh[5] showed that indol and tyrosine pro-

[4] Longcope, W. T., *Jour. Exper. Med.*, 1913, xviii, 678.

[5] Woolley, P. G., and Newburgh, L. H., *Jour. Am. Med. Assn.*, 1911, lvi, 1796. Newburgh and Woolley, *Lancet-Clinic*, 1912, N.S., lxviii, 404.

duced no remarkable changes. In this series we have shown that egg-albumen, casein, and albumen give practically the same negative results whether or not chloroform is administered with them.

In all our experiments we have used comparatively small doses (at any rate they were not massive); because if, as has been suspected, such anatomic conditions as chronic interstitial nephritis are the result of absorption from the gastro-intestinal tract, then the quantities of materials absorbed are probably small, and the process covers a considerable period of time. Perhaps we have not continued our work over a long enough period. However that may be, there is evidence that the living proteins in the form of bacteria are far more potent than the non-living, and that even the less pathogenic ones may produce greater damage in the length of time covered in our experiments than has been occasioned by the substances we have used.[6] These substances (microorganisms) are entering the system continuously, and since we know that they produce, often rapidly, anatomic changes, a series of experiments such as ours is chiefly of value in proving the relative lack of danger from absorption of the substances which we have employed,—at least in normal animals.

SUMMARY.

We have attempted to discover whether or not certain protein materials, such as albumose, casein, and albumen, when introduced parenterally (peritoneally) into experimental animals, are able to produce organic lesions. These proteins were used alone or in combination with chloroform, which was administered in oil and as an anesthetic. Our results were negative.

[6] Opie, *loc. cit.*